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AUTHOR'S ERRATA AND EMENDATIONS

Page 90, figure 2, B, "stem leaves" should be "rosette leaves," 2, H, "(a) *L. graminifolia*" should be "(a) *L. tatarica*" and "(c) *L. tatarica*" should be "(c) *L. graminifolia*."

Page 170, table 11, in title and column headings, "F_3" should be "F_2."

Page 210, table 9, subheadings, "50 hours" should be "71 hours" and "73 hours," should be "97 hours."

Page 251, column of figures for correlation coefficients, insert a minus before ".112" and ".388."

Page 264, legend of plate 10, line 5, "position" should be "positive."

Page 302, literature cited, No. 9, line 2, "1852" should be "1867"; line 3, insert "Ed. 5" after "Studirende," and change "637" to "749."

Pages 448 and 449, tables 9 and 11, all braces in these tables should be eliminated.

Page 460, last line, "best F_3" should be "best F_4."

Page 473, table 3, column headed "Upper quartile fiber length (inches)," line 3, "0.159" should be ".0159."

Page 513, table 6, "recovered" in heading should read "eliminated."

Page 581, table 1, third column, last line, insert "12.00" in place of leaders.

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CERTAIN ENVIRONAL AND NUTRITIONAL FACTORS AFFECTING APHANOMYCES ROOT ROT OF GARDEN PEA[1]

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INTRODUCTION

The *Aphanomyces* root rot (*Aphanomyces euteiches* Drechs.) has been an important disease of pea (*Pisum sativum* L.) in the canning crop in Wisconsin and numerous other States for the past 20 years or more. Earlier studies by Jones and associates (*9, 10*)[2] and by members of the New Jersey Agricultural Experiment Station (*6; 13, Rpt. 52*) emphasized the importance of soil moisture as well as temperature in their influence upon the development of the disease. More recently Walker and Musbach (*20*) found little or no infection in the greenhouse in soil from a severely diseased field when the moisture level was kept low while at higher moisture contents the disease was very severe.

Efforts to find a practicable control for this disease have been only partially successful. Investigators at the New Jersey Station in a series of reports (*5, 11, 12, 13, 14*) have shown that heavy applications of complete fertilizers are effective in reducing the severity of infection. They also found that the nitrogen compounds used in the fertilizer mixtures were much more influential in reducing root rot than the phosphorus and potassium components. No benefit resulted when the fertilizer was applied after infection had taken place. Walker (*19*) in Wisconsin and Geach (*3*) in Australia have obtained similar decreased infection with the use of complete fertilizers. Walker and Musbach (*20*) showed that applications of fertilizer containing nitrogen reduced root rot damage in the field somewhat more than corresponding applications without nitrogen. They also found that greater protection was effected when the fertilizer was applied in the drill at the time of planting than when it was applied in a furrow at one side of but removed a slight distance from the seed.

The purpose of the present investigation was to examine more closely the relation of certain environmental factors to the development of the disease, and to study the effect of mineral nutrition on its incidence and severity.

MATERIALS AND METHODS

In the study of the effect of nutrition on the disease a constant-flow sand-culture method was used. The nutrient solutions were allowed to drip at the rate of 600 to 800 cc. per day into 8-inch flowerpots filled

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[2] Italic numbers in parentheses refer to Literature Cited, p 19

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with washed, sterilized silica sand. The inside surface of the pot was varnished to prevent diffusion of salts. Each pot was equipped with an intermittent siphon which allowed the nutrient solution to rise within 2 inches of the surface before draining. The time required for each pot to fill and drain varied from 24 to 36 hours.

The nutrient solution used throughout was a modification of that described by Hoagland and Snyder (7). In addition to the "normal" solution, solutions containing extra quantities of one of the elements N, P, and K and solutions lacking one of these elements were used. The ratio of each element in the "excess" solutions to that in the normal solution was approximately: N, 1.15 to 1; P, 2.0 to 1; and K, 1.28 to 1. A trace of each of the essential minor elements was added to all solutions. The reaction of all solutions was maintained between pH 5.5 and 6.2. The composition of the various nutrient solutions used is given in table 1. In the experiments involving higher concentrations of the nutrients, NaCl was added so that the proportions of the major elements could be varied without changing the total salt concentration.

TABLE 1. *Chemical composition of the nutrient solutions at the basic concentration used in the sand-culture experiments*

Chemical	Cubic centimeters of molar solutions used to make up 10 liters of nutrient						
	Normal	+K	−K	+P	P	+N	−N
$Ca(NO_3)_2$	[1] 50.0	50.0	50.0	50.0	50.0	50.0	
$CaCl_2$							50.0
KNO_3	50.0	50.0		50.0	50.0	50.0	
KCl		16.7			10.0		50.0
$NaNO_3$			50.0			22.2	
NaCl [2]	22.2	5.5	22.2	12.2	22.2		22.2
$MgSO_4$	20.0	20.0	20.0	20.0	20.0	20.0	20.0
KH_2PO_4	10.0	10.0		10.0		10.0	10.0
NaH_2PO_4			10.0	10.0			

[1] 50 cc. of a M/1 salt solution in 10 liters equals 0.005 M concentration.

[2] Not used in the dilute nutrient solution experiments.

Inoculum was obtained by growing the organism in pea decoction in 250 cc. Erlenmeyer flasks. About 25 wrinkled-type pea seeds were placed in each flask, 100 cc. of water was added, and the flasks were plugged and autoclaved for 2 hours. Each flask was inoculated with a fragment of an agar culture of the fungus, and the organism was allowed to grow for 10 to 12 days. The zoospore suspension was obtained by removing the mycelial mats, washing them first in running tap water for 2 hours, and rinsing them in distilled water. They were then placed in shallow pans with just enough water to cover them. Abundant zoospore formation followed within 6 to 8 hours.

Inoculation was made by adding 100 cc. of the zoospore suspension to each pot. In the first experiments the siphon drains were plugged at the time of inoculation and the sand was flooded with the nutrient solution. After 24 hours the plugs were removed and the nutrient was allowed to flow through in the normal manner. In the experiments in which the higher salt concentrations were included, small 1 mm. bore siphons were placed over the edges of the pots after the sand was saturated and the nutrient was allowed to continue dripping into the pots. With the ends of the small siphons placed under the sand, the siphon column was not broken and the nutrient solution continued to flow into the pots without causing them to flood. The pots were drained after 24 hours.

The Wisconsin Perfection variety of pea was used throughout all experiments, except where indicated. Eighteen seeds were planted in each pot.

DISEASE SYMPTOMS AND METHOD OF DISEASE RATING

In sand cultures the first symptoms of the disease appear on the roots within 4 or 5 days after inoculation. Water soaking spreads above and below the initial infection zone without appreciable discoloration in the area of infection. The water-soaked tissue is firm at first, but it gradually becomes yellowish and soft, darkening with age and eventually collapsing and disintegrating. About 4 days after the underground symptoms may be found, water soaking appears in the stem above the sand level and progresses an inch or more up the aerial portion of the stem.

The extent of disease development was recorded when all or nearly all plants in the dilutest solution in the respective series showed severe above-ground symptoms. Plants were removed from the sand and divided into five arbitrary classes based on the severity of disease and designated as follows: 0, No disease; 1, slight water soaking on epicotyl or on primary or secondary roots; 2, moderate water soaking of primary root or epicotyl, with or without slight darkening of the infected tissue, which remains firm; 3, infected areas extensive, darkened, and becoming soft, but without collapse of tissue; 4, extensive water soaking and discoloration with collapse and disintegration of part or all of the infected tissue; plants dying previous to inspection were rated in this class.

A disease index calculated on the basis of the above classes appeared to be a more accurate indication of the extent of infection than the percentage of infected plants, since it afforded a means of indicating the relative severity in combination with the percentage of plants affected. The index was calculated by using the class figures as weighted values. To obtain the index the number of individuals in each class was multiplied by the class number, the sum of the products of each class multiplied by 100, and this figure divided by 4 times the total number of plants. Thus, when all the plants were healthy, the rating was 0, and when all plants showed severe infection, the rating was 100, while intermediate grades were represented by intermediate index figures.

ENVIRONMENTAL STUDIES

TEMPERATURE IN RELATION TO GROWTH OF THE FUNGUS

Plates of potato-dextrose agar adjusted to a neutral reaction were inoculated in the center with 5-mm. disks from the periphery of an actively growing 4-day-old Petri-dish culture of *Aphanomyces euteiches*. Four replicates were placed at each of eight temperatures ranging in 4° intervals from 8° to 36° C., inclusive. Increments of radial growth were measured daily.

The results are shown graphically in figure 1. The greatest daily increase and total colony growth occurred at 28°. No growth occurred at either extreme temperature. From the minimum to the optimum there was a steady increase in rate of growth with increase in temperature. Above 28° the growth rate dropped rapidly. The straight-line trends in the graph indicate that growth was not retarded by the accumulation of staling or other metabolic products of the fungus.

REACTION OF MEDIUM IN RELATION TO GROWTH OF THE FUNGUS

The reaction series was set up by using various quantities of H_3PO_4, and of mono-, di-, and tri-sodium phosphate solutions, each with 0.1 cc. of concentrated H_2SO_4 added, according to the method described by Tilford (*16*). The quantity of phosphates for each pH level was 20 cc.; the amount of each fraction was varied to produce the desired pH level. Each 20-cc. phosphate mixture and a 200-cc. quantity of potato-dextrose agar (1.7 percent agar) was sterilized separately and thoroughly mixed just before pouring the plates. One plate of each pH level was melted, diluted with three parts of distilled water, and the pH determined with a glass electrode. The plates were inoculated in the same manner as in the temperature series. Increments of growth were measured daily and the curve of growth plotted after 4 days.

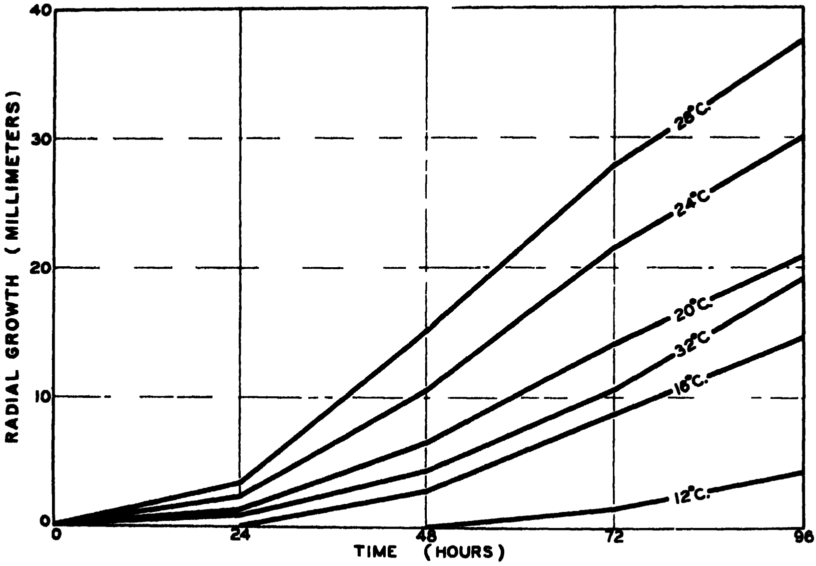

FIGURE 1. The effect of temperature on growth of *Aphanomyces euteiches* on potato-dextrose agar.

The results are shown graphically in figure 2. The fungus exhibited a tolerance to a wide range of acidity, with the limits at about pH 3.4 and slightly above pH 8.0. A broad optimum range from about pH 4.5 to 6.5 occurred, with an apparent isoelectric point at pH 5.9. The daily increment of growth at each pH value remained quite constant, indicating no apparent drift in the reaction of the medium or any interference of staling or other metabolic products of the fungus.

EFFECT OF SOIL TEMPERATURE ON INFECTION

The soil-temperature range at which root rot infection can occur has been found by Jones and Drechsler (*9*) to lie between 10° and 30° C., with an optimum somewhere between 15° and 30°. Further study of this important environmental factor was undertaken to determine more exactly the rate of disease development at different soil temperatures. Twenty-four soil-temperature cans were filled to within 1½ inches of the top with clean, sterile silica sand, and each can was equipped with a siphon for drainage purposes. Fifteen seeds were planted in each can and watered with a balanced nutrient solution of one-tenth the salt concentration described in table 1. All cans were placed at a temperature of approximately 24° in order to secure uniform plant growth. Every 3 days these were drained and fresh nutrient was added. Two days before inoculation four cans were placed in each of six soil-temperature tanks in which the temperatures were maintained at approximately 12°, 16°, 20°, 24°, 28° and 32°, so that the sand in each case did not vary more than 1°. The air temperature varied between 20° to 24°.

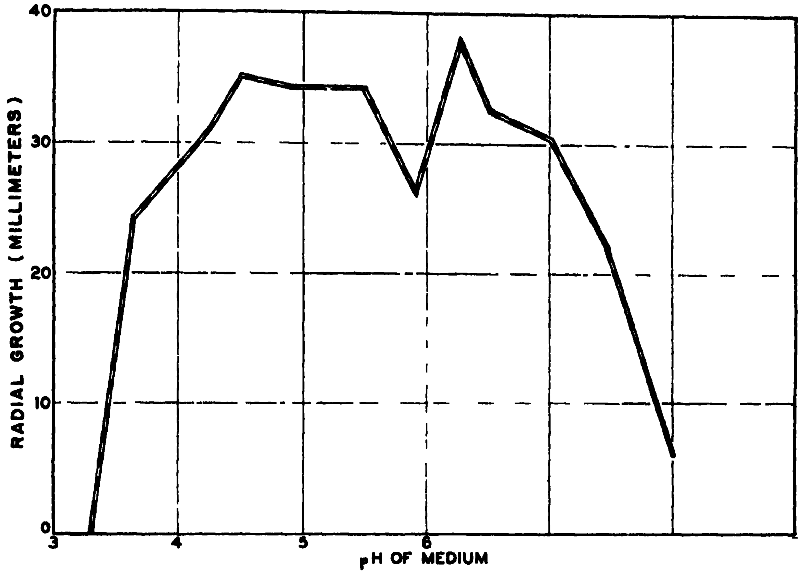

FIGURE 2. The effect of initial pH of potato-dextrose agar on growth of *Aphanomyces euteiches*.

Fourteen days after planting, each can was saturated with nutrient and 100 cc. of a zoospore suspension were added. The cans were then partially drained in order to draw the inoculum around the roots, but sufficient moisture was left at the surface for infection to take place. Notes were taken in the manner described for the nutrient studies.

In the 24° and 28° tanks infection was visible 4 days after inoculation. The experiment was terminated 11 days after inoculation when nearly all plants at these two temperatures were infected. The results (table 2) show a definite optimum for disease development at 24° and 28°. No infection was found on plants at 12°, while at 16° some infection did occur. The rate of disease development at the various sand temperatures appeared to parallel quite closely the rate of growth of the organism on agar.

TABLE 2.—*The effect of sand temperature on Aphanomyces root rot development as shown by the extent of disease development 11 days after inoculation*

Disease index at the temperature (°C.) indicated					
12°	16°	20°	24°	28°	32°
0	12.5	51.6	90.4	86.4	55.6

EFFECT OF SOIL MOISTURE ON INFECTION

Others (*5, 9, 10, 20*) have noted that root rot infection is most severe when the soil moisture is high. Observations in Wisconsin during the past three years (1938, 1939, 1940) have indicated that the disease has been more severe in the seasons of abundant rainfall.

For the purpose of obtaining more information on the effect of soil moisture on root rot, an experiment employing two moisture levels was made. The soil was taken from a naturally infected field near Clyman, Wis. It was mixed thoroughly and two portions were adjusted to 45 and 75 percent of the water-holding capacity, respectively. The saturation point of the soil was reached when the moisture content was 43 percent of its dry weight. To maintain a fairly constant moisture content, Wisconsin soil-temperature cans watered every third day were used. A 2-inch layer of ground cork was placed above the soil to reduce evaporation from the surface. Six replicates were included at each moisture level and the arrangement of the cans was randomized. The cans were kept at 20° to 25° C. after 15 seeds had been planted in each. The plants were dug for inspection 30 days later.

TABLE 3.—*The effect of high and low soil moisture on Aphanomyces root rot infection as shown by the extent of disease development 30 days after planting*

[Cans kept at 20° to 25° C. after 15 seeds had been planted in each]

Soil moisture in percentage of water holding capacity	Percentage of plants infected	Disease index
45	[1] 1.4	0.7
75	72.0	52.7

[1] 70 plants in 6 replicates
82 plants in 6 replicates

The results are shown in table 3. A statistical analysis of the results was not made because of the extreme difference in the amount of infection between the two series. It is evident from both the disease index and the percentage of infected plants that the higher moisture markedly favored disease development. At 45-percent water-holding capacity, there was a very small amount of infection, indicating that this moisture content of the soil is approximately the minimum at which infection can take place in this soil. This is somewhat higher than the results of Haenseler (*5*), who found that the minimum for infection occurred at about 30 percent of the water-holding capacity of the soil he used, when inoculated by means of a zoospore suspension. Although not determined experimentally, a somewhat higher moisture level than 45 percent of the water-holding capacity could undoubtedly have been maintained in soil used in the present investigation without severe infection.

NUTRITIONAL STUDIES

Although the beneficial action of fertilizers in reducing root rot has been demonstrated in infected soil, the nature of this action has not been explained. Carpenter (*2*) and LeBeau (*8*) showed that high nitrogen increased the root rot of sugarcane caused by *Pythium* spp., and that adequate phosphorus decreased it. Vanterpool (*17, 18*) demonstrated that the browning root rot of wheat occurred in phosphorus-deficient soil and that applications of phosphorus reduced its severity. Broadfoot and Tyner (*1*), studying the *Helminthosporium* root rot of wheat under controlled conditions, observed that any treatment which resulted in the production of undernourished plants favored disease development. The work of these investigators indicates that unbalanced nutrition predisposes sugarcane and wheat to the causal organisms concerned. Workers at the New Jersey Experiment Station (*11, Rpt. 57*) in field studies over a period of several years found that increase in yield of peas on root-rot-infested soil was almost directly proportional to the amount of fertilizer applied even when the rate was as high as 2,400 pounds per acre. This was interpreted as indicating that the control of root rot was a contributing factor to higher yield in addition to the direct effect of nutrients on plant growth, especially at the high fertilizer levels. Because a more adequate explanation of the relation of host nutrition to root rot development in peas was desirable, further investigation of this question was undertaken.

RELATION OF SOIL FERTILITY TO DISEASE DEVELOPMENT

In order to obtain a further check on the effect of nitrogenous fertilizers, a field test was made in 1940. A 1-acre area was chosen in the field just mentioned in which root rot had caused a complete crop failure the previous year. Each plot was 24 by 100 feet in dimension; each treatment was made on three replicates arranged in randomized order. The treatments consisted of an unfertilized check, 2 12-6 fertilizer at the rates of 333, 534, 760, and 1,108 pounds per acre, respectively, and 0 12 6 at the rate of 1,094 pounds per acre. The 2 12 6 fertilizer was a commercial preparation, and the 0 12 6 mixture was compounded from Tennessee Valley Authority superphosphate and commercial 50-percent K_2O. The fertilizers were applied broadcast on recently prepared soil and harrowed in promptly about 2 weeks before planting. Profusion variety was planted on May 8 by means of a seed drill. Throughout the season rains were frequent and the soil was thus kept at a high moisture content, a condition which favored root rot development.

On June 12 random samples, each containing from 40 to 50 plants, were dug from each plot and rated on the same scale as was used in the sand-nutrient series. Twelve days later, on June 24, a second sample, and on July 9 a third sample of plants was dug and rated. The results of the first two inspections were analyzed statistically and are given in table 4.

TABLE 4. *Effect of fertilizer treatments on naturally infested soil in the field on the development of Aphanomyces root rot on Profusion peas sown on May 8*

Fertilizer treatment	Rate of application in pounds per acre	Mean disease index	
		35 days after planting	47 days after planting
None		63.70	97.50
2-12-6	333	43.50	97.50
2-12-6	534	45.53	94.53
2-12-6	760	28.50	89.93
2-12-6	1,108	21.20	81.30
0-12-6	1,094	46.57	92.80
Minimum significant difference (5-percent point)		20.77	5.82

The results at 35 days show clearly that disease development in the unfertilized check was significantly greater than that which occurred when 2-12-6 fertilizer was applied at the rates of 760 and 1,108 pounds per acre. At the lower rates of 2-12-6 and at the 1,094-pound application of 0-12-6 there was a little less disease than in the unfertilized plots, but the difference was not significant. A comparison of the 0-12-6 fertilizer application with the corresponding 2-12-6 treatment shows a significant difference in favor of the nitrogen-bearing fertilizer. At 47 days the root rot had increased markedly and the decrease in disease at the 760- and 1,108-pound treatments with 2-12-6 was barely perceptible. At the time of the third field inspection all plots were completely and severely infected, although even at that time the plants in the plots receiving 1,108 pounds of 2-12-6 fertilizer were visibly taller and greener than those in the other plots. The crop was so poor in all plots, however, that yields were not taken. Although from the standpoint of commercial control the results were disappointing, the trend was nevertheless in general agreement with those previously observed (*3*, *5*, *11*, *12*, *13*, *14*, *19*, *20*).

DILUTE NUTRIENT-SOLUTION EXPERIMENTS

In the first series of sand-culture experiments the previously described solutions were applied at one-tenth the basic concentration. In addition to the solutions lacking N, P, and K, a solution lacking S was also employed. Although the nutrient solutions used were quite dilute, it was hoped that the degree of variation in the balance between the major elements would show whether the incidence of root rot was influenced by the nutrition of the host plant.

Four pots were used for each nutrient. Two weeks after planting all were inoculated with a zoospore suspension of *Aphanomyces euteiches* of an undetermined spore concentration. At the end of the first experiment the series was repeated. Inoculation this time was made 3 weeks after planting. In both experiments, above-ground symptoms appeared within 7 days and all plants were dead within 14 days after inoculation. Regardless of the nutrient employed, infection was equally rapid and severe.

Peas were again planted in the pots used in the second experiment without sterilizing and washing the sand. In this case the inoculum was largely in the form of oospores in the numerous fine roots left in the sand when the previous crop of infected peas was removed, rather than the introduced zoospores which brought about the initial infection. The seedlings emerged normally, but infection was visible 5 days after emergence. All plants were dead within 12 days. Again no effect of nutrient variation was discernible.

On the assumption that the form of nitrogen might have an influence on the disease, one experiment was set up to determine the effect of NH_4 ions as compared with NO_3 ions. The NO_3 solutions was the same as the normal solution used in the experiments described above. In the NH_4 solution the $Ca(NO_3)_2$ and KNO_3 were replaced by $CaCl_2$ and KCl, respectively, and an equal number of nitrogen atoms was added in the form of NH_4Cl. The pots were inoculated 2 weeks after planting. Disease development and severity were equal in both nitrate and ammonium solutions, indicating that at the nutrient concentrations used, neither form of nitrogen had any effect on disease development.

From the foregoing experiments, it was evident that with the nutrient concentration employed, the development and severity of the disease were independent of the nutrient-ion balance, or of the two types of nitrogen ions.

RELATION OF INCREASED NUTRIENT CONCENTRATION AND ION BALANCE TO ROOT ROT INFECTION

With the failure of the low-concentration experiments to show any influence on root rot, it became obvious that the fertilizer action was dependent upon factors other than nutrient-ion balance alone. Since the previous experiments had been made at a very low concentration of nutrients, it seemed advisable to determine whether differences resulting from varied nutrient-ion balance might not occur at higher concentrations. In order first to observe the effect of increased nutrient concentrations, only the complete nutrient solution was used.

In the previous experiments the basic solutions (table 1) were diluted to one-tenth concentration. The concentrations hereafter are referred to as multiples of the basic solution. Thus, the dilute solution mentioned above is designated as 0.1H, the basic as 1H, double concentration as 2H, etc. The arrangement of the sand cultures was the same as in the previous experiments. The inoculation technique used has already been described, and in the following experiments the concentration of the inoculum was standardized at 7,500 to 8,000 zoospores per centimeter of inoculum. One pot in each group of four was left uninoculated.

In the first experiment normal nutrient solution at concentrations of 0.1H, 1H, 2H, and 3H were used. Thus, the highest concentration was 30 times as strong as the nutrient used in the previous sand-culture experiments. As soon as infection appeared it became evident that at the highest nutrient concentration there was a distinct decrease in amount of disease as compared with that in the 0.1H nutrient concentration (fig. 3). The plants were dug for inspection when all the

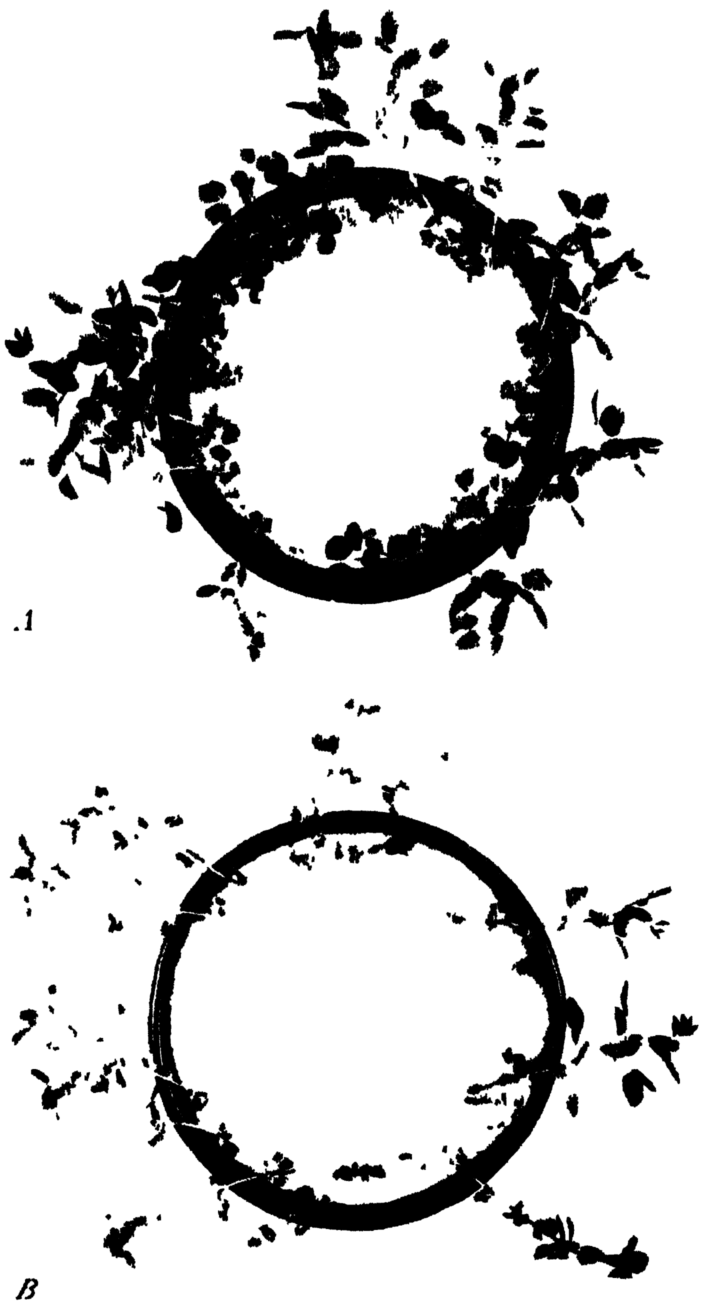

FIGURE 3.—Pea plants 18 days after inoculation with *Aphanomyces euteiches*. *A*, Plants grown in a high concentration of nutrient solution (3H). *B*, plants grown in a low concentration of nutrient solution (0.1H). The plants in *B* are all severely diseased and most have already died; of those in *A* only one showed any above-ground signs of disease at the time of the photograph.

plants in the 0.1H nutrient showed above-ground disease symptoms. The results (fig. 4, experiment 1) show a striking decrease in the amount and severity of root rot infection at the higher nutrient concentrations. The decrease in the severity appeared in direct proportion to the concentration of the nutrient.

In order to verify the findings in the above experiment, another series was run. Again the same relation between the severity of disease and the concentration of nutrients resulted (fig. 4, experiment 2). Since the 3H solution did not completely control the disease, however, still another experiment was made in which the concentrations 0.1H, 1H, 2H, 3H, 4H, and 5H were used. The same relative decrease in disease with increased concentration occurred. At the two higher concentrations, 4H and 5H, no infection whatsoever could be found (fig. 4, experiment 3; fig. 5). In this experiment the infection was not so severe in any of the nutrients, although the experiment was allowed to run about 10 days longer than the first two. For this reason the possibility remains that a small amount of infection might take place at the 4H and 5H concentrations. However, from the results of this experiment and the general relation found between concentration and infection in all three experiments, it is highly probable that the zero point for infection, under the conditions of these experiments, would be found at a concentration between 3H and 5H.

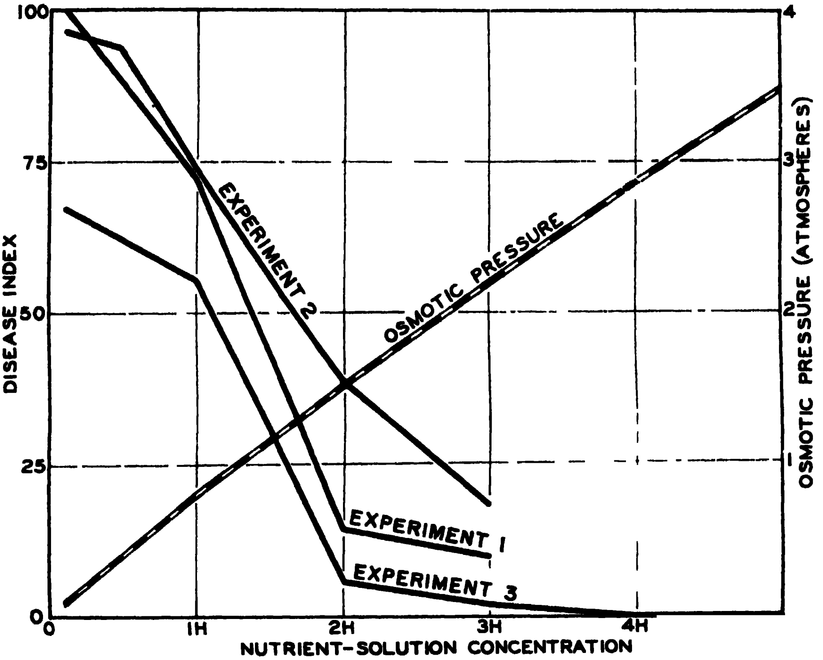

FIGURE 4. The relation of salt concentration to osmotic pressure of the nutrient and to the development of *Aphanomyces* root rot of pea. Representative plants from experiment 3 are shown in figure 5. Explanation of nutrient-solution concentration symbols will be found in the text.

The marked reduction in the amount and severity of root rot with increase in concentration of the solution again raised the question of the relation of the nutrient elements to the disease. Although the relative proportions of all elements in the normal solution were the

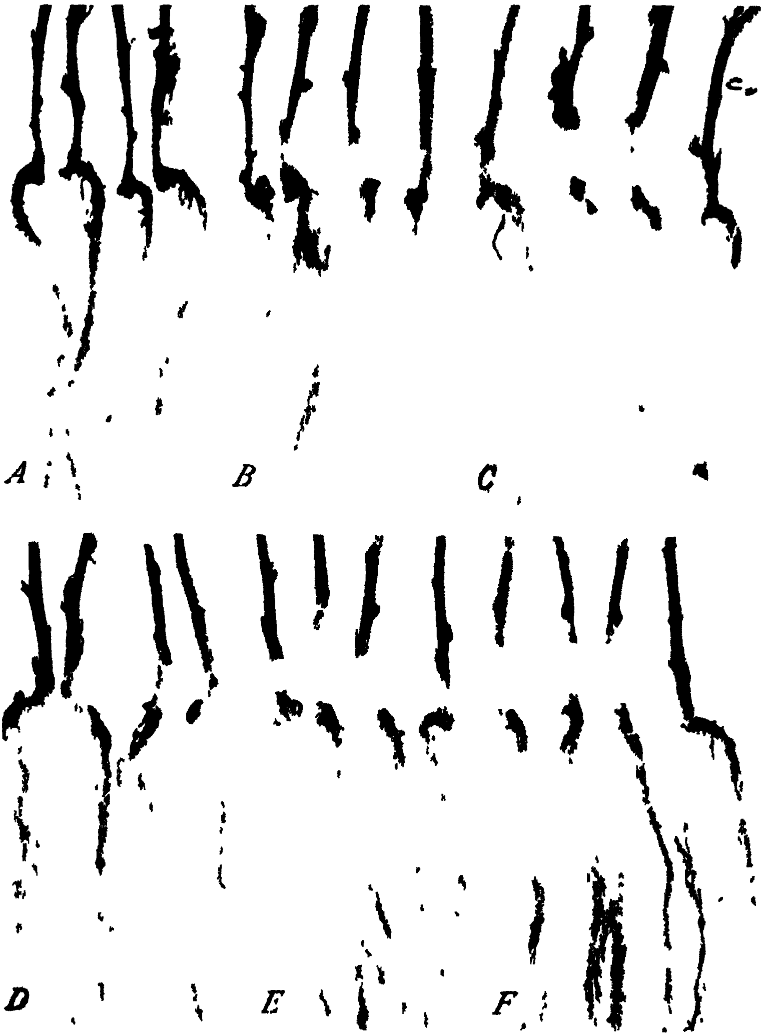

Figure 5.—The relation of salt concentration of the nutrient solution to the development of *Aphanomyces* root rot of peas grown in silica-sand culture. See experiment 3 in figure 4. Photographed 27 days after inoculation. The concentrations used were as follows: *A*, 0.1H; *B*, 1H; *C*, 2H; *D*, 3H; *E*, 4H; *F*, 5H (see further explanation in the text). Plants in *A* are severely diseased; many fibrous roots have been killed and cortical decay of the upper taproot and the lower stem is evident. In *B* root decay is moderate and there is considerable cortical decay in the stems. In *C* decay in the root and stem is slight. In *D* there is very slight root and stem discoloration. In *E* and *F* no signs of disease were found.

same, the possibility that one of the major elements was the effective agent still existed. In order to determine whether this was the case, each of the three elements N, P, and K was varied in the same proportions as in the experiments in which the low concentration was used. This was done at each of the three concentration levels 1H, 2H, and 3H. With this range any effect of a single element should be detected readily. With the exception of the experiment with varying nitrogen at the 1H level, a 0.1H check was run with each experiment for purposes of comparison. The results are shown in table 5.

TABLE 5.—*The effect of varied proportions of nitrogen, potassium, and phosphorus at different nutrient concentration levels on the development of Aphanomyces root rot of pea, as indicated by the disease index*

Relative level of ion being varied	N varied at concentration[1]			K varied at concentration[1]			P varied at concentration[1]		
	1H	2H	3H	1H	2H	3H	1H	2H	3H
High	86.0	3.1	3.1	39.1	3.4	0.7	33.7	1.9	0.0
Normal	61.2	3.2	.6	11.0	3.2	.6	30.1	3.2	.6
Minus	77.2	3.3	.7	13.8	8.0	.7	35.6	0	0
0.1H control		82.1	82.1	75.0	82.1	81.4	84.4	82.1	81.4

[1] See text for explanation of symbols indicating nutrient-solution concentration.

On the basis of the previously discussed field experiments made by other investigators, and the one made by the authors, it was expected that the quantity of nitrogen in the nutrient would have considerable influence on the severity of the root rot. The results of these experiments indicate clearly that the variations in the amount of nitrogen used, from none to 15 percent more than in the normal solution, had little or no effect on the degree of infection at any of the three nutrient concentrations. At each concentration level, nitrogen-deficiency symptoms were clearly visible in the series lacking nitrogen before the experiments were terminated, eliminating the possible influence of nitrogen carried in the seed. Since the disease development was so rapid in the lower nutrient levels, there seems to be no reason to believe that the food stored in the seed had any effect on the results of any experiment.

Although the field experiments have shown very little reduction in root-rot damage by the potassium and phosphorus portions of the fertilizers, these two elements were tested in sand culture at the same nutrient concentrations as the nitrogen portion. The results failed to show any influence of high or low amounts of either element as compared with the normal solution on the amount of disease at any of the three nutrient concentrations (table 5).

At the three nutrient levels used in the experiments on elements in varied proportions the same general decrease in disease occurred with increased concentrations, as shown in the experiments involving increased concentrations of the normal nutrient solution. However, it must be kept in mind that at any given nutrient concentration, the total salt concentration of the nutrient solutions remained essentially constant, regardless of the lack or excess of any one element. It is evident, from the results of this set of experiments, that development of the disease is not dependent upon an adequate nutrient balance, but that the reduction in disease is directly correlated with the increased concentration of the nutrient salts in the root zone of the host plants.

White and Ross (*21*) in a study of the effect of fertilizer salts have found the nitrogen components of present-day fertilizers to be much more active in raising the salt concentration of the soil solution than equivalent amounts of salts of potassium and phosphorus. It seems probable, then, that the favorable action of the nitrogen-bearing fertilizers in reducing root rot in the field is due to the greater activity of the nitrogen fractions of the fertilizer in increasing the salt concentration of the soil solution.

In order to determine the osmotic pressure of the various nutrient concentrations used, the freezing point depression method was employed. A Drucker-Burian microthermometer was used, and with the equation $P = 12.06\Delta - 0.021\Delta^2$, the osmotic pressure, P, in atmospheres was calculated.[3] The values obtained are shown in figure 4. It is possible that these values could be used as a guide in soil experiments designed to determine the fertilizer requirements for field control of root rot.

The results of the above experiments substantiate the suggestion made by workers at the New Jersey Agricultural Experiment Station (*11, Rpt. 59*) that the increased fertilizer concentration of the soil was responsible for reduced disease when fertilizers were applied. The observations made by Walker and Musbach (*20*) that less root rot developed when a complete fertilizer was placed with the seed than when it was placed away from the seed at the time of planting would seem to be explained by the fact that a greater fertilizer concentration is present at the zone of major root development when the fertilizer is placed with the seed.

EFFECT OF INCREASED NUTRIENT CONCENTRATION AFTER INOCULATION

The investigators at the New Jersey Station (*13, Rpt. 55; 11, Rpts. 56, 57, 59; 14, Rpt. 58*) have found that fertilizers must be applied before infection has occurred in order to be effective in reducing root rot damage. An experiment was carried out to determine the effect on incidence of root rot of increasing the salt concentration from a low to a high level at definite intervals of time after inoculation. The purpose was to find the length of time over which infection takes place under ideal conditions in the sand culture, and to observe whether the increased concentration of nutrients would decrease disease development after infection had taken place. Eight rows of four pots each were started with a normal solution at the 0.1H concentration and one row at the 3H concentration. Two weeks after planting, three pots in each row were inoculated while the fourth was left uninoculated. One row of the 0.1H concentration and the one at the 3H concentration were continued throughout the experiment. Plants in a second row of 0.1H concentration were removed periodically to determine progress of the disease. Of the other six rows, the first was changed to 3H 1 day after inoculation, the second after 2 days, etc. When the 3H solution was applied, all pots in the row were flushed with this solution in order to bring the concentration of the nutrient in the pots to the high level immediately. The results are shown graphically in figure 6.

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[3] This equation is from Gortner (*4, pp. 317-338*) $\Delta = \Delta' - 0.0125\Delta' U$, when Δ is the difference in °C between the freezing point of the solution and that of pure water, while U represents the difference in °C between the freezing point and the point of undercooling at which crystallization occurs.

Daily inspection of the roots of plants in the 0.1H solution disclosed no visible signs of infection until 4 days after inoculation. However, delay of the rise in nutrient concentration for 2 days after inoculation resulted in a marked increase in infection, while delay until the fourth day resulted in little reduction in disease. Thus the shift in concentration of nutrient to a level that would otherwise prevent severe disease development was of no avail if made after infection had occurred. The results of this experiment are in agreement with the field observations at the New Jersey Station, in which

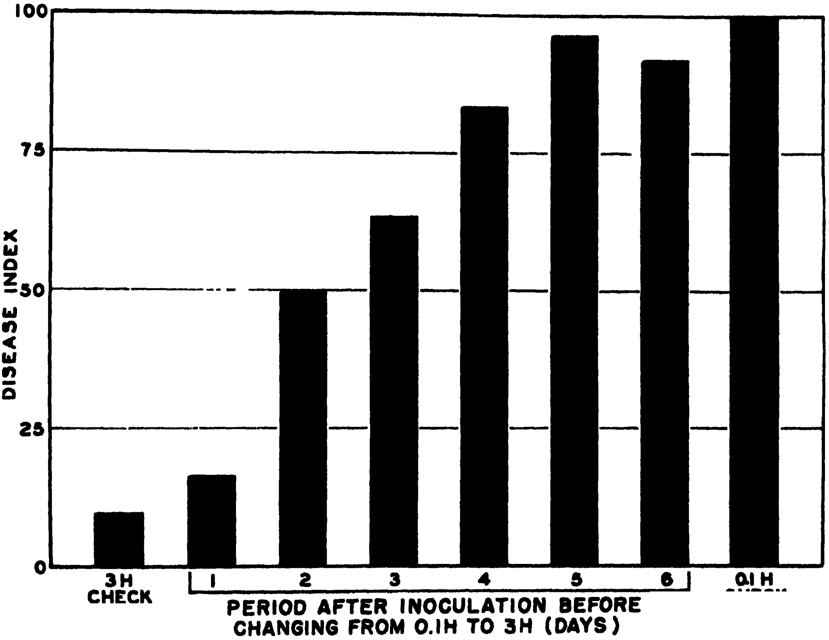

FIGURE 6. The development of root rot (in terms of disease index) in plants grown at high (3H) and low (0.1H) concentrations of nutrient solution, compared with those started at 0.1H and shifted to 3H at various intervals after inoculation. Note the steady increase in the disease index as the interval between the inoculation and the shift to the high-concentration nutrient was increased.

the beneficial effects of fertilizer did not prevail if the fertilizer was applied after infection had taken place (*13, Rpt. 55*).

EFFECT OF NUTRIENT CONCENTRATION ON GROWTH OF THE FUNGUS

The direct inhibiting effect of high salt concentrations of the nutrient solution on the growth of the organism would seem to be a possible explanation for decreased severity of the root rot disease. In order to determine the effect of the nutrient concentration on the growth of the fungus, agar plates of the normal solutions at the 0.1H, 1H, 2H, 3H, 4H, and 5H levels were poured and inoculated with the fungus. The agar was prepared by making up the nutrient at double strength and combining with an equal volume of 4 percent agar. The ingredients were sterilized separately and mixed just prior to the pouring of plates. At the higher concentrations 3 to 4 drops of M/1 NaOH per 200 cc. of agar solution were added when necessary to bring the reaction to pH 6.0 or 6.2. The separate sterilization of agar and nutrient solutions was found necessary when previous attempts to sterilize them after mixing had resulted in a strongly acid reaction in the more concentrated nutrients.

The plates were inoculated in the center by 2-mm. disks of agar taken from the periphery of a vigorously growing 4-day-old culture of *Aphanomyces euteiches*. Six plates were used at each nutrient concentration. Daily measurements of radial growth were made. Because of the absence of all organic and other nutrient materials, except for that occurring in the agar, growth was slow and sparse. Nevertheless, growth did occur at the higher nutrient concentrations at approximately the same rate as in the lower, except at the 5H level at which slightly reduced growth was observed. From these results it is apparent that the growth of the organism is not inhibited at the nutrient concentrations at which no infection occurred in the sand cultures.

Geach (*3*) made a brief study of the growth of *Aphanomyces euteiches* in cultures containing various nitrogen compounds. He found growth of the organism to be retarded on pea-decoction agar containing any one of the following compounds: 0.5 percent urea, 1.1 percent NH_4NO_3, and 1.4 percent $NaNO_3$. On Shear's corn-meal agar containing 2.8 percent $NaNO_3$ no growth occurred. The apparent contradiction between these results and those of the authors is readily explained when the total salt concentrations of the sand-culture nutrient solutions and the agar cultures employed by Geach are noted. The osmotic values for the different concentrations of nutrient solutions are shown in figure 4. The calculated osmotic values for the 1.4 percent $NaNO_3$ in the pea-decoction agar used by Geach is approximately 6.8 atmospheres. This is roughly twice the osmotic value of the 5H solution employed in the sand-culture studies, and far in excess of that at which disease control was obtained. Evidently the salt concentration of the 2.8 percent $NaNO_3$ was sufficiently high to prevent growth of the organism. From the previously discussed nutrient studies, it has been shown that the presence or absence of nitrogen has no effect on the severity of root rot. Thus the inhibitory action of nitrogen in some form, as implied by the work of Geach, cannot be considered as the true cause for the control of pea root rot.

DISCUSSION

The *Aphanomyces* root rot of pea is a widespread and often destructive disease of this crop. Up to the present time, the environmental relations and disease control studies have been confined, for the most part, to field observations and experiments. Disease development is favored by high soil moisture, and occurs within a temperature range of about 10° to 30° C. Studies on the control of this disease, made largely by Haenseler at the New Jersey Agricultural Experiment Station and confirmed by other investigators, have shown that applications of complete fertilizers to infested soil tend to reduce root rot. It has been suggested that the nitrogen fraction in the fertilizer is the most important one in the reduction of disease.

The root rot organism in potato-dextrose-agar culture grows at temperatures from 12° to 32°, inclusive, with an optimum at 28°, while the disease develops in plants grown in inoculated sand maintained at constant temperatures from 16° to 32° with an optimum at 24° and 28° C. No infection was found at 12°, although Jones and Drechsler (*9*) with experiments in soil, involving a longer period of time, found a small amount of infection at 10°. The rate of disease development in sand and the rate of growth of the organism on agar approximate each other quite closely at the various temperatures used.

In naturally infected soil practically no root rot occurred when the moisture was maintained at 45 percent of the water-holding capacity of the soil, while at 75 percent of the water-holding capacity 72 percent of the plants were infected. The soil-moisture relationship found here is in agreement with the observations of other investigators. Since the experiment was not terminated until a month after planting, it would appear probable, under certain field conditions, that a single or an occasional heavy rain would not result in complete root rot infection, but rather that a prolonged condition of high soil moisture is required to produce this condition. This, of course, could happen in poorly drained fields, or under conditions of frequent heavy rainfall, as was the case in Wisconsin in 1940 when root-rot damage was widespread. This is further supported by the results of the 1940 fertilizer trial, for, on June 12, 35 days after planting, 37.8 percent of the plants in the unfertilized check plots were still free from root rot, although rainfall had been abundant. On June 24, after a 12-day period of almost continuous rainfall, all plants were infected, but 6.8 percent were still classed as slightly diseased.

During 1940 the fertilizer trial, mentioned above, was made on a field known to be heavily infested with the root rot organism. Applications of 2-12-6 fertilizer were made at various rates up to 1,108 pounds per acre. One treatment of 0-12-6 at 1,094 pounds per acre was also included. The two highest applications of 2-12-6 (760 and 1,108 pounds per acre) resulted in significantly less disease than in the unfertilized plots. Lesser rates of 2-12-6 and the 1,094-pound rate of 0-12-6 were ineffective in decreasing root rot damage. Since the phosphorus and potash content of the two treatments (2-12-6 at 1,108 pounds, and 0-12-6 at 1,094 pounds) were the same, it is evident that these two components are less active in reducing root rot infection than is the nitrogen fraction. The rainfall during this season was extremely heavy; had there been less rain, it is probable that the degree of control would have been greater in the plots receiving nitrogen in the fertilizer.

When the nutrient solution was used in continuous-flow sand-culture experiments at the concentration described by Hoagland and Snyder (*7*), and in concentrations up to five times that described, infection and disease severity decreased in direct proportion to the increase in the concentration of the nutrient solution. When severe infection occurred in the one-tenth dilution, only a small amount occurred at three times the basic concentration, and none whatsoever occurred when the nutrient concentration was raised to four and five times the basic concentration.

At the basic concentration and at two and three times that concentration, solutions each having N, P, and K in excess, and lacking each of these elements were tested. The total salt concentration was maintained constant at each concentration level. The same relation as previously described was observed. The disease decreased as the total salt concentration of the nutrient solution increased; the favorable or unfavorable balance of any of the three essential elements in the nutrient solution in no way modified the disease response.

This apparent disagreement with the results of the fertilizer investigations appears to be explained when the effects of the various fertilizer fractions on the concentration of the soil solution are investigated. White and Ross (*21*), as a result of their study of the effects of fertilizers on the salt content of the soil solution, have found that the nitrogen compounds $NaNO_3$, $(NH_4)_2SO_4$, and NH_4NO_3 increase the salt concentration of the soil solution much more than corresponding quantities of the potassium and phosphorus salts now used in commercial fertilizers. If this is the case, the greater degree of control obtained with nitrogenous as compared with nonnitrogenous fertilizers could probably be attributed directly to the greater increase in the salt concentration of the soil solution by the nitrogen fractions of the fertilizers than by the potassium and phosphate fractions. The possible detrimental effect of nitrogen alone on the organism is ruled out for the reason that the disease development was equally inhibited in nutrient solutions of high salt concentration whether nitrogen was present in excess or lacking entirely.

Under favorable conditions for infection in the sand culture, all plants may become infected within 5 days after inoculation. After infection has occurred, and before any but the slightest macroscopic symptoms are visible, changing the solution concentration from a low level to one sufficiently high to largely inhibit infection, if done at the outset, does not delay the development of the disease within the host plant. For this reason it would appear that the action of the high concentration of the nutrient salts is that of preventing infection, either by direct action of some kind on the organism, or by rendering the host more resistant. In the latter case the promotion of host resistance is much less pronounced after infection has taken place.

On agar made from nutrient solutions ranging from one-tenth to five times the basic concentration, the organism appeared to grow equally well. Only a slight depression in growth rate was noted at the highest nutrient concentration. Therefore it appears likely that the mechanism by which the root rot is prevented when in the presence of high salt concentrations is due to some other cause than concentrations of nutrient salts inhibitory to the organism.

A possible explanation for the lack of root rot at the high nutrient concentrations is to be found in the work of Nightingale and Farnham (*15*). In a study of the effect of the nutrient concentration on the anatomy of the sweet pea (*Lathyrus odoratus* L.) they have found the roots of plants growing in dilute solutions to be highly succulent and lacking in mechanical strength. The roots of plants grown in high nutrient concentrations, on the other hand, were typically woody, mechanically strong, and distinctly lacking in succulence. A similar condition occurred in the stem tissue. Since the range in nutrient concentration employed by them (0.5 to 3.0 atmospheres osmotic pressure) closely paralleled that used in the present investigations (0.12 to 3.5 atmospheres), it may possibly be, in the case of pea root rot, that the host plants acquire a morphological resistance as a direct result of the high concentration of the nutrient when grown in such solutions.

SUMMARY

The investigations comprise a study of temperature, moisture, and nutrition in relation to root rot (*Aphanomyces euteiches* Drechs.) of peas.

On potato dextrose agar the most rapid radial expansion of the organism occurred at 28° C. No growth occurred at either 8° or 36°. The optimum temperature for disease development on plants grown in sand was found at 24° and 28°. No infection was noted at 12° during an 11-day period, while nearly all plants at the optimum temperature were severely affected in that period.

On phosphate-buffered potato-dextrose agar, the pH limits for growth were about pH 3.4 and slightly above pH 8.0. The optimum, as measured by radial expansion of the organism, occurred between pH 4.5 and 6.5. An apparent isoelectric point appeared at pH 5.9.

In infested soil, practically no infection occurred when the moisture was maintained at 45 percent of the water-holding capacity. At 75 percent of moisture-holding capacity, infection was quite severe.

Under conditions of controlled nutrition in a continuous-flow sand culture, inoculated by means of a zoospore suspension, the severity of disease decreased in direct proportion to the increase in total salt concentration of the nutrient solution. No infection occurred at the highest concentrations employed, while all plants were severely diseased at the lowest concentration. Varying the ratio of each of the elements, N, P, and K from complete absence to an excess of that in the balanced solution had no effect on disease development, whether in dilute or concentrated nutrient solutions.

When conditions are favorable in the sand culture, all plants may become infected within 5 days. Once infection takes place, high nutrient concentrations do not appear to inhibit the development of the disease.

On agar cultures made from the nutrient solutions employed in the sand culture, the organism grew readily on the concentrations at which infection was prevented in the sand culture.

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INHERITANCE OF A MELANINLIKE PIGMENT IN THE GLUMES AND CARYOPSES OF BARLEY[1]

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INTRODUCTION

Although black barleys are rarely grown commercially in the United States, they have been used in hybridization for many years. Many valuable characters, including smooth awns, have been transferred from varieties with black hulls and caryopses. A large number of varieties and strains of black barley have been introduced or selected from crosses involving black and white color. The factor pair responsible for the production of black pigment in barley has been studied by a number of workers, but no reference has been made to the various degrees of black coloration that exist. This paper presents the results of a study of the inheritance of the black or melaninlike pigment that occurs in various intensities in the glumes and caryopses of barley (*Hordeum* spp.) seed in some 8,000 F_3 progenies from more than 50 crosses.

REVIEW OF LITERATURE

The character black vs. white flowering glumes has been reported to be controlled by a single factor pair in several publications reviewing genetic studies in barley. Black has been considered completely dominant over white, giving F_2 ratios of 3 black to 1 white. Tschermak (*13*)[2], Biffen (*1*), Griffee (*4*), Hayes et al. (*6*), Ubisch (*14*), Hor (*7*), Robertson (*10*), Sigfussen (*12*), Buckley (*2*), and Kuckuck (*9*) have all reported monogenetic ratios of black vs. white caryopses or glumes. Harlan (*5*) reported the black color to be the result of a melaninlike pigment found in the lemma, palet, and pericarp only, and the color in the aleurone to be due to anthocyanin pigments. A special study of color inheritance in barley and possible linkage relationships has been reviewed and discussed by Buckley (*2*). He concluded that black pericarp is always associated with black lemma, that either the same gene is responsible for the coloring in both the lemma and the pericarp or that very close linkage between two separate genes must exist. No linkage was found between the genes controlling black vs. white glume and pericarp color and any of the anthocyanin factors. The genes for black and white color were reported by Buckley to be inherited independently when tested with long vs. short rachilla

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[2] Italic numbers in parentheses refer to Literature Cited, p 28

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hairs, hooded vs. awned, and hulled vs. hulless. Gaiser (*3*) had previously reported that black lemma was linked with the texture of awn, rachilla hairs, color of caryopsis, one albino factor, and one factor for the resistance to *Helminthosporium*. Hor (*7*) reported black lemma linked with texture of awn and length of rachilla hairs. Buckley (*2*) found three linkage groups and in addition suggested that the factor pairs black vs. white and hulled vs. naked caryopsis represented two additional linkage groups. More recent work by Robertson, Wiebe, and Immer (*11*) indicates that the factor pair black vs. white is in linkage group No. 2 along with $A_t\, a_t$, an albino factor found in Trebi I. They list a factor responsible for the production of a third outer glume in the same linkage group with black vs. white lemma. Ivanova (*8*) describes this third outer glume as a new character in barley. Seed of this particular barley has not been available to workers in the United States, so that the genes controlling black vs. white and $A_t\, a_t$ are the only genes in linkage group No. 2 available to them.

Sigfussen (*12*) has pointed out that the black pigment does not express itself until just before ripening, making it difficult to classify immature plants.

No reference was found in the literature to studies of crosses between colored parents that differed in the intensity of black pigment in the lemma and caryopsis.

MATERIAL AND METHODS

A number of varieties and strains containing anthocyanin and the melaninlike pigment were used in this study.[3] A few white-glumed varieties thought by some workers to produce occasional dark types also were included in an observational nursery. These varieties have been grown for periods of 7 to 9 years. Head selections also have been made from them, some of which appeared to show greater color variation than others.

After preliminary studies all of the varieties were arbitrarily classified into five groups based on the intensity of the melaninlike pigment. Two of the varieties in each group breeding most uniformly for color intensity were chosen for the genetic studies. These groups were designated as (1) dense black, (2) black, (3) medium black, (4) gray, and (5) white.

Although the climatic conditions were generally favorable for color classification, it was difficult in some crosses. Attempts were made to extract the black pigment so that the extracts might be used as a basis for classifying color, but with no success.

Most of the more than 100 black-glumed varieties and strains were found to be relatively stable and uniform for color intensity. A few varieties such as Blackhull had previously been reported to contain some individuals with lighter colored glumes and caryopses than the variety as a whole. In a study of a large number of head rows of Blackhull (C. I.[4] 878), a light-colored type was isolated and found to breed true. This light-colored strain of Blackhull (C. I. 878) was used as one of the parent strains in the gray group. A number of the color variants found in the original Blackhull variety were definitely identified as mixtures or hybrid segregates. A few other varieties, including C. I. 875 and 3204, contained two or more color classes which, when isolated, have continued to breed true for dark and light glumes, respectively. There is no evidence from a 9-year study that any of the varieties grown under Utah conditions have thrown mutants of either lighter or darker color intensity than was found in the original material after pure color types were established.

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[3] Obtained from H. V. Harlan, principal agronomist, in charge of barley investigations, Division of Cereal Crops and Diseases, Bureau of Plant Industry.

[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases.

The varieties used in this study to represent the five color-intensity groups are shown in table 1, and some of them are illustrated in figure 1. Group 1, arbitrarily classed as dense black, is characterized by its

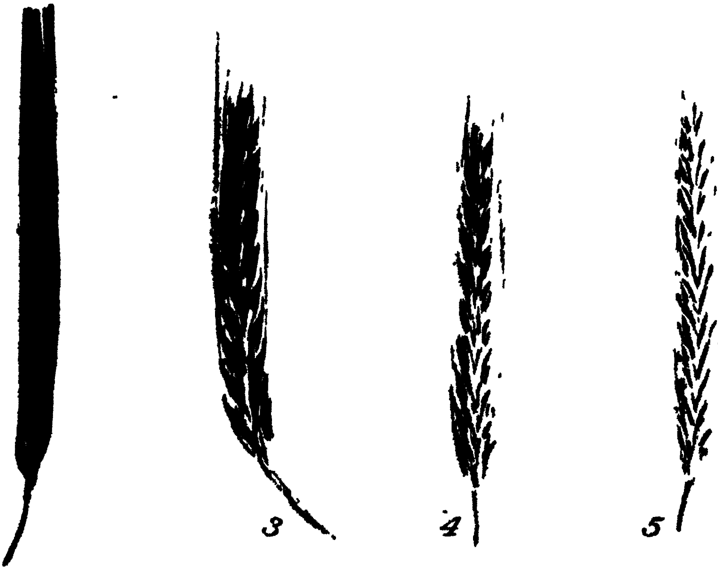

FIGURE 1. Spikes of barley illustrating four of the five color-intensity groups: Group 1, dense black (*BB*), group 3, medium black ($B^{mb}B^{mb}$); group 4, gray (B^gB^g), and group 5, white (*bb*)

extreme black pigmentation in the flowering glumes, pericarp, and awns. Blackhull (C. I. 878) and Black Algerian (C. I. 708) are representative of this group. Group 2, classed as black, appeared to be slightly lighter in color intensity than group 1, the awns often showing a yellowish color. Bucher (C. I. 1461), Donjon (C. I. 1264), an unnamed variety (C. I. 3910 1), and Jet (C. I. 2222) are listed as representative varieties in group 2. Group 3, described as medium black, shows slightly less pigmentation than group 2. The lateral florets are generally light-colored in the two-rowed and intermediate segregates found in this group. C. I. 4376 and 2970 are representative varieties. Group 4 is best described as gray in color and generally shows sufficient contrast to the other groups to be easily identified. A light-colored type isolated from Blackhull (C. I 878) and Dentil (C. I. 1260) was chosen to represent this group. In group 5 are included varieties with white glumes and pericarp, of which C. I. 4354 and Blackhull 1178 (C. I. 5679) are representative.

All combinations among 2 of the above varieties from each of the 5 color classes were made. The total of 45 crosses involved combinations of all fertility classes such as two-rowed, six-rowed, intermedium, and deficiens, as well as other character differences. Other crosses also were made for additional information on the inheritance of black color.

TABLE 1. *Color-intensity groups and descriptions of the varieties selected to represent them*

Group and variety	C. I. No.	Varietal characters			
		Glume and pericarp color	Awn color	Fertility type of lateral florets	Caryopsis
1, Dense black					
Blackhull	878	Dense black	Black	Deficiens	Hulled
Black Algerian	708	do	do	Vulgare	Do
2, Black					
Jet	*2222*	Black (lateral florets gray)	Dark to light	Intermedium	Hulless
Bucher	1461	Black	do	Vulgare	Hulled
Unnamed	3910-1	Brown to black	do	do	Hulless
Donjon	1264	Black	do	do	Hulled
Medium black					
Unnamed	4376	Dilute black	Gray	Deficiens	Do
Do	2070	Dilute black (laterals yellow)	do	Distichon	Do
4, Gray					
Blackhull (light colored)	878	Gray	Colorless	Deficiens	Do
Dentil	1266	do	do	Distichon	Do
5, White					
Unnamed	4354	White	do	Vulgare	Do
Blackhull selection 1178	5679	do	do	Intermedium	Do

Experience indicated that observations on the primary spikes were more reliable than those on spikes chosen at random and that all material should be thoroughly ripened before harvest in order to classify color density accurately. This latter fact also was pointed out by Sigfusson (*12*).

All crosses were studied through the F_3 generation as a check on F_2 classifications. Numerous doubtful types were continued in head rows for 2 or 3 years longer or until they could be identified more positively. Normal color densities failed to develop in seasons of excessive moisture or cloudy weather. Such seasons sometimes necessitated the study of large populations in the F_4 and F_5 generations in order to determine the breeding behavior of the F_2 plants.

The material was space-planted in rows 8 to 17 feet long. A large part of the material was studied in the field.

EXPERIMENTAL RESULTS

COLORED × WHITE

Pigmented varieties representing the four color-intensity groups were crossed with varieties having white or colorless glumes and pericarps. In each case F_2 ratios of 3 black to 1 white resulted, as is shown in table 2. This is in agreement with the ratios generally obtained by previous workers. No intermediate color classes could be found, regardless of the intensity of the black parent used.

TABLE 2. *F_2 segregations in crosses between colored and white varieties*

	Genetic symbols	F_2 segregation based on F_3 breeding behavior		Deviation / Standard error
Dense black × white	$BB \times bb$	Dense black	White	
Blackhull × Blackhull selection 1178		230	75	0.17
Blackhull × C. I. 4354		340	113	.03
Black Algerian × C. I. 4354		211	80	.97
Black Algerian × Blackhull selection 1178		156	64	1.10
Black × white	$BB \times bb$	Black	White	
C. I. 3910.1 × C. I. 4354		527	185	.61
Bucher × C. I. 4354		205	71	.28
Jet × C. I. 4354		354	124	.50
Donjon × C. I. 4354		110	46	1.29
C. I. 3910.1 × Blackhull selection 1178		257	93	.68
Jet × Blackhull selection 1178		153	72	2.43
Donjon × Blackhull selection 1178		51	21	.82
Medium black × white	$B^{mb}B^{mb} \times bb$	Medium black	White	
C. I. 2970 × C. I. 4354		262	99	1.25
C. I. 2970 × Blackhull selection 1178		194	83	1.91
Gray × white	$B^gB^g \times bb$	Gray	White	
Blackhull gray segregate × C. I. 4354		216	79	.71
Dentil × C. I. 4354		253	96	1.08
Dentil × Blackhull selection 1178		326	126	1.41
White × white	$bb \times bb$	No segregation.		
Blackhull selection 1178 × C. I. 4354				
		3,844	1,427	

It should be noted that the white segregates are in excess in all but two of the crosses. In all crosses studied the deviation in favor of light-colored segregates is still more pronounced, giving 6,166 dark to 2,234 light, with a deviation of 134±40. No satisfactory explanation has been found for this behavior.

COLORED × COLORED

Tabulated results of the segregations from crosses of colored × colored are shown in table 3.

DENSE BLACK × DENSE BLACK

No indication of any segregation could be observed in the F_2 and later generations of crosses between dense black and dense black varieties.

DENSE BLACK × BLACK

Six crosses of dense black × black were studied, but classification was difficult. These two color groups differ so slightly in intensity of coloration, which expresses itself largely in the awns, lateral florets, and culms, that they may be included in one group.

DENSE BLACK × MEDIUM BLACK

With favorable seasonal conditions the segregating generations from the cross of dense black × medium black could be classified. A single-factor difference explains the results obtained. Varieties or segregates of the six-rowed type consistently have a slightly lighter coloration than those of the non-six-rowed types. When the color classes of the parents were similar, classification of the segregates was more difficult in the progeny from crosses involving six-rowed by non-six-rowed varieties than when both parents were of like fertility.

TABLE 3.—*F_2 segregations in crosses between pigmented varieties representing the different color-intensity groups*

Cross	Genetic symbols	F_2 segregation based on F_3 breeding behavior		Deviation / Standard error
Dense black × dense black	$BB \times BB$			
Blackhull × Black Algerian		No segregation		
Dense black × black	$BB \times BB$			
Blackhull × Bucher		No segregation		
Blackhull × Jet		do		
Black Algerian × Jet		do		
Black Algerian × C. I. 3467		do		
Blackhull × C. I. 3910-1		do		
Black Algerian × C. I. 3910-1		do		
Dense black × medium black	$BB \times B^mB^m$	Dense black	Medium black	
Blackhull × C. I. 2970		176	72	1.47
Black Algerian × C. I. 2970		Difficult to classify		
Dense black × gray	$BB \times B^gB^g$	Dense black	Gray	
Blackhull × C. I. 1260		215	80	.84
Black Algerian × Blackhull gray segregate		176	70	1.25
Black Algerian × Dentil		71	23	.29
Black × black	$BB \times BB$			
Donjon × C. I. 3910-1		No segregation		
Jet × C. I. 3910-1		do		
C. I. 4363 × Jet		do		
Jet × Bucher		do		
Black × medium black	$BB \times B^mB^m$			
Jet × C. I. 2970		Difficult to classify		
C. I. 3910-1 × C. I. 2970		do		
Black × gray	$BB \times B^gB^g$	Black	Gray	
Bucher × Blackhull gray segregate		187	67	.51
C. I. 3910-1 × Blackhull gray segregate		228	87	1.07
Donjon × Blackhull gray segregate		125	43	.18
C. I. 3467 × Dentil		166	42	1.60
Bucher × Dentil		188	57	.63
C. I. 3910-1 × Dentil		215	68	.38
Medium black × medium black	$B^mB^m \times B^mB^m$			
C. I. 2970 × C. I. 4376		No segregation		
Medium black × gray	$B^mB^m \times B^gB^g$	Medium black	Gray	
C. I. 2970 × Blackhull gray segregate		305	117	1.29
C. I. 4376 × Dentil		91	28	.52
C. I. 2970 × Dentil		173	53	.54
Gray × gray	$B^gB^g \times B^gB^g$			
Blackhull gray segregate × Dentil		No segregation		

DENSE BLACK × GRAY

Crosses involving dense black × gray were extremely clear-cut in their segregation. A monofactorial condition was suggested by all such crosses.

BLACK × BLACK

No indication of segregation was observed in any of the four crosses studied involving black × black. In C. I. 3910-1, a hulless variety, the pericarp was more densely colored at full maturity than the glumes.

BLACK × MEDIUM BLACK

Differences in the parents chosen for black and medium black could be seen when conditions for color development were favorable. Crosses involving these two groups, although indicating segregation, did not permit clear-cut separation in the F_2 or later generations.

BLACK × GRAY

Each of the six crosses studied indicated a single-factor difference between the color intensities black and gray. In seasons when bleaching was abnormal it was necessary to continue considerable material into the F_4 or F_5 generation in order to separate the classes.

MEDIUM BLACK × MEDIUM BLACK

No segregation was evident when two varieties of this color-intensity group were crossed.

MEDIUM BLACK × GRAY

Crosses between varieties of the medium black and the gray classes yielded monofactorial segregations in the F_2 generation.

GRAY × GRAY

A cross between C. I. 1260 × Blackhull (C. I. 878) (gray) showed no indication of segregation in later generations.

DISCUSSION

Although five color-intensity groups were described and studied, the problem would have been simplified if the group listed as black had been eliminated or combined with dense black. Great difficulty was encountered in classifying the plants in crosses involving black × dense black or black × medium black. It appears that the black group may differ slightly from either of these other dark-colored groups and that possibly still other color groups might be found by a positive method of determining color intensity. The medium-black group appears to be distinguishable from the other groups but is not too well established as yet.

Using only data from crosses in which the segregates could be classified by inspection, the results definitely establish three color groups, the dense black (*BB*), gray (B^gB^g), and white (*bb*). The factors responsible for the production of the melaninlike pigment appear to represent an allelomorphic series, since only monofactorial ratios were obtained from any combination showing color-intensity differences. In all crosses the darker color class showed complete dominance.

These studies also suggest that certain other varieties might give more clear-cut results in crosses.

The Dentil variety displayed a winter-grown habit in two seasons, which complicated the studies, but early seeding eliminated further difficulty in this respect.

Buckley (*2*) concluded that black pericarp is always associated with black lemma and that either the same gene is responsible for the coloring or close linkage exists. No segregates exhibiting recombinations of glume and pericarp colors were obtained in these experiments.

SUMMARY

No mutation or new off-color type was observed during a study of more than 100 varieties and strains of barley, although in some varieties individual plants segregated for two color classes.

Barley varieties were grouped into five color classes, based upon the intensity of the black melaninlike pigment in the glume and pericarp. These groups were (1) dense black, (2) black, (3) medium black, (4) gray, and (5) white. More than 50 crosses involving all group combinations were made and studied in the segregating generations. Eliminating group 2 (black), all crosses among the remaining four groups yielded monofactorial segregations in the F_2 generation. More refined classification might establish one or more additional color intensities.

The medium-black group appeared to be distinct from the others, but its relationship could not be established.

The data suggest an allelomorphic series of factors causing various color-intensity expressions. The factors definitely established are black (*BB*), gray (B^gB^g), and white (*bb*). The denser color was always completely dominant over the lighter one.

Despite attempts to isolate other color groups from the progeny of certain crosses, the parental types only were recovered in the homozygous condition in later generations.

Pigment formation in both the pericarp and the flowering glume appears to be controlled by a single series of allelomorphic genes.

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A STUDY OF THE C FACTOR AS A REQUIREMENT FOR GROWTH OF HEMOPHILUS GALLINARUM [1]

By J. P. DELAPLANE, *associate poultry husbandman*, and H. O. STUART, *poultry husbandman, Rhode Island Agricultural Experiment Station*

INTRODUCTION

The so-called C factor requirement for the growth of *Hemophilus gallinarum* [2] was reported by Kessens,[3] who observed that chicken serum heated at 90° to 100° C. for 10 minutes supported growth of *H. influenzae*, but failed to support growth of the fowl coryza bacillus, *H. gallinarum*.

Delaplane et al.[4] found that some factor in chicken-blood serum was adversely affected by boiling but was not completely destroyed, since some growth occurred when the boiled serum was used at the base of nutrient agar slants. For convenience, they also referred to this factor as the C factor.

Because of the incomplete destruction of the factor in boiled blood serum, the question arose as to the possibility that the physical changes brought about by boiling might have prevented the organism from utilizing some necessary nutrient and that the effect observed was not the result of chemical action.

METHODS

To avoid the firm type of coagulated proteins such as result from boiling serum or diluting serum in broth, it was decided to add the sterile chicken-blood serum in the ratio of 1 part of sterile serum to 3 parts of a sterile 2-percent sodium citrate normal salt solution. Such a solution is alkaline, and it was thought that the metaproteins resulting from boiling would remain in suspension. This proved to be the case, as the serum solution merely became cloudy after boiling or after heating for 15 minutes at 15 pounds' pressure in the autoclave.

Serum solutions thus prepared were boiled for 5, 10, and 15 minutes, rapidly cooled, and used with nutrient broth and at the base of nutrient agar slants for cultural tests. Serum solutions similarly prepared were autoclaved and tested in the same manner. Sterile chicken-blood plasma instead of serum was also used in the tests.

As boiling in the manner described did not appear to destroy the growth properties of the serum or plasma for *Hemophilus gallinarum*, boiled preparations of serum and plasma were centrifuged, the supernatant filtered through filter paper, and the filtrate sterilized by passing through Swinny bacteriological filters.

Unheated serum and plasma filtered in this manner were known to support growth of *Hemophilus gallinarum*, and so were not employed.

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[2] Kessens refers to this organism as *Haemophilus coryza*.

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Two different strains of *Hemophilus gallinarum* and a smooth strain of *H. influenzae* were used for testing the boiled serum and plasma solutions, and a bacteriological filtrate was obtained from them.

RESULTS

The results of the various tests are shown in table 1. The boiled serum and plasma solutions supported good growth of *Hemophilus gallinarum*, whereas the autoclaved preparation failed to do so. This would seem to indicate that boiling the serum or plasma does not destroy the factors necessary for growth; and that the possible destruction of the factor in serum as reported by Kessens[5] and by Delaplane et al.[6] resulted from physical changes in the medium.

The results of filtering the boiled preparations would seem to indicate that the growth factors are tied up with the coagulation proteins since the filtrates failed to support growth.

The tests in which *Hemophilus influenzae* was used showed that it, too, would grow in the boiled citrated serum and plasma preparations, but not in the bacterial filtrates of the same material.

It may be possible that through the action of enzymes or other factors *Hemophilus influenzae*, unlike *H. gallinarum*, is able to utilize the growth factors of the more firmly coagulated forms of the boiled chicken serum proteins (as reported by Kessens [5]), but no studies of this kind have been attempted.

As a result of these studies, the writers believe the C' factor of Kessens [5] to be one of a physical rather than of a distinctly chemical or nutrient nature, and thus the cultures of *Hemophilus gallinarum* used in the present work require only the X and V factors for growth rather than the X, V, and C' factors.

TABLE 1. *The effects upon the growth of Hemophilus gallinarum and H. influenzae of different methods of treating preparations of 25-percent chicken-blood serum or or plasma, in 2-percent sodium citrate normal salt solution*

Treatment of chicken blood preparation and method of use	Type of growth (as indicated by stained preparations)	
	H. gallinarum [1]	H. influenzae
Serum boiled 5 minutes, used at base of agar slants	Good growth	Good growth
Serum boiled 5 minutes, used with broth	Growth	Growth
Serum boiled 10 minutes, used at base of agar slants	Good growth	Good growth
Serum boiled 10 minutes, used with broth	Growth	Growth
Serum boiled 15 minutes, used at base of agar slants	Good growth	Good growth
Serum boiled 15 minutes, used with broth	Growth	Growth
Serum autoclaved 15 minutes, used at base of agar slants	No growth	No growth
Boiled serum Swinny filtrate at base of agar slants	do	Do
Plasma boiled 5 minutes, used at base of agar slants	Good growth	Good growth
Plasma boiled 5 minutes, used with broth	Growth	Growth
Plasma boiled 10 minutes, used at base of agar slants	Good growth	Good growth
Plasma boiled 10 minutes, used with broth	Growth	Growth
Plasma boiled 15 minutes, used at base of agar slants	Good growth	Good growth
Plasma boiled 15 minutes, used with broth	Growth	Growth
Plasma autoclaved 15 minutes, used at base of agar slants	No growth	No growth
Boiled plasma Swinny filtrate at base of agar slants	do	Do

[1] No differences were noted between the 2 strains of *H. gallinarum* used in the tests

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[5] KESSENS, B. H. See footnote 3.

[6] DELAPLANE, J. P., ERWIN, L. E., and STUART, H. O. See footnote 4.

THE DISTRIBUTION OF POTASSIUM IN BRIGHT LEAF CIGARETTE TOBACCO AND ITS INFLUENCE ON THE QUALITY OF THE LEAF[1]

By M. F. Gribbins, *assistant in agricultural and biological chemistry*, J. J. Reid *assistant professor of bacteriology*, and D. E. Haley, *professor of soils and phytochemistry, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

Numerous workers have reported that potash fertilization of leaf tobacco is favorable both to the yield and quality of the crop. Many fundamental studies have been conducted on the absorption and translocation of potassium by growing plants and its accumulation within the various cells and tissues. The relation of this element to various biochemical and physiological phenomena has received due consideration.

The present investigations involve a study of the influence of various quantities of potash fertilizers applied to growing plants in the field on the absorption and localization of potassium within the plants and the influence of this element upon their quality. For these studies, samples of tobacco were obtained from certain of the experimental plots at Chatham, Va., where the Virginia Agricultural Experiment Station is conducting field tests on this crop. Samples of both the 1938 and 1939 crops were taken in December from a barn where the mature leaves had been stored in the usual manner after harvesting and curing. The results of studies conducted on similar samples of the 1937 crop have been reported (*2*).[2]

REVIEW OF THE LITERATURE

Within the growing plant potassium may be found in the form of compounds which are quite soluble in water. It is readily transported throughout the plant and is usually found to accumulate in regions of active growth. Nightingale, Schermerhorn, and Robbins (*6*) found that the greater part of the potassium in tomato plants was soluble in water and, when present in limited quantities, was translocated from regions of less intensive to regions of more intensive metabolism. If a potassium-deficient plant was allowed to fruit, the potassium was translocated to the fruit at the expense of all other portions of the plant, which resulted in its death. Confirmatory results have been reported by James and Penston (*3*) and by Janssen and Bartholomew (*4*). The last-named workers found that leaf starvation in the tomato plant due to a lack of potassium was progressive, and extended from the lower to the upper leaves. Dead leaves taken from the plants showed a much lower content of potassium than actively growing leaves, indicating a reutilization of this element in metabolism. It is apparent, therefore, that a plant should contain ample quantities of

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[2] Italic numbers in parentheses refer to Literature Cited, p. 38

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potassium for its normal growth and metabolism, and that there should be a fair distribution of this element throughout the plant.

MATERIALS USED AND METHODS OF ANALYSIS

The samples were taken from plots each of which received 30 pounds of nitrogen, 80 pounds of phosphoric acid, and various quantities of potash per acre as shown in table 1. Supplementary treatments involved the application of magnesia and limestone to all plots at the rate of 200 pounds per acre. Two percent of the potash was applied as muriate, the remainder as the sulfate. Representative samples [3] of leaves were taken from the lower, the middle, and the upper third of the stalks. Each sample represented two grades, good quality and scrap quality. In 1938 only the good quality grades were analyzed.

Methods used for chemical analysis involved the removal of the midrib and grinding the remainder in a Wiley mill. Total nitrogen was determined by the Kjeldahl method modified to include the nitrogen of nitrates, potassium by the platinic-chloride method according to Thomas et al. (*10*), and moisture by drying at 97.5° C. for 2 hours in a drying oven. Reducing materials were determined on an aqueous extract of the sample by the Qusumbing and Thomas method (7) and calculated as glucose. Samples were extracted with water at 50° C. for 2 hours and then overnight at room temperature. The samples were clarified, deleaded, made up to volume, filtered, and the reducing power determined on aliquots.

TABLE 1.—*Fertilizer treatment of the experimental tobacco plots studied*[1]

Plot No.	Row treatment 1,000 pounds per acre of indicated formula	Side dressing of K_2O per acre[2]	Total K_2O in treatment per acre
		Pounds	*Pounds*
1	3-8-0	0	0
2	3-8-3	0	30
3	3-8-6	0	60
4	3-8-6	30	90
5	3-8-6	60	120
6	3-8-6	100	160
7	3-8-6	140	200
8	3-8-6	240	300

[1] Data presented in this table were obtained from E. M. Matthews, superintendent of the Chatham substation of the Virginia Agricultural Experiment Station.

[2] Side dressings were applied on either side of the row about 8 inches from the plants 3 weeks after transplanting.

PRESENTATION OF DATA

Samples of both the 1938 and 1939 crops were analyzed for potassium, nitrogen, and reducing materials. The reducing materials, to a large extent, are made up of fermentable carbohydrates. A large portion, but by no means all, of these materials is made up of glucose and related sugars.

The 1938 crop produced good yields and commanded a fair price on the market (table 2), notwithstanding the fact that it was grown under conditions of excessive rainfall and was somewhat damaged by hail. The analyses made on the best quality samples are presented in table 3.

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[3] These samples were submitted through the courtesy of E. M. Matthews, superintendent of the Chatham substation.

TABLE 2.—*Effect of various fertilizer treatments on the yield and value of the 1938 and 1939 crops of bright leaf tobacco produced on the experimental plots studied*

1938 CROP

Plot No.[1]	Yield per acre	Value per acre	Price per pound
	Pounds	*Dollars*	*Cents*
1	758	140.20	18.5
2	1,096	218.08	19.9
3	1,168	239.70	20.5
4	1,192	254.35	21.3
5	1,188	233.63	19.7
6	1,222	241.09	19.7
7	1,198	222.74	18.6
8	1,228	237.53	19.3

1939 CROP

Plot No.[1]	Yield per acre	Value per acre	Price per pound
1	888	120.40	13.6
2	1,096	150.80	13.8
3	1,112	155.60	14.0
4	1,104	162.00	14.7
5	1,084	147.60	13.6
6	1,120	156.40	14.0
7	1,148	143.60	12.5
8	1,076	144.80	13.5

[1] See table 1 for formulas and quantities of fertilizers used on these plots

The 1939 crop was grown under dry conditions early in the season but later the rainfall was satisfactory. On the whole, however, this crop was somewhat inferior to that of the 1938 crop (table 2).

Chemical analyses of the tobacco for both years are presented in table 3, all results being reported on a moisture-free basis. From the results as listed in table 3, the ratio of nitrogen to potassium was calculated. The results are presented in figures 1 and 2.

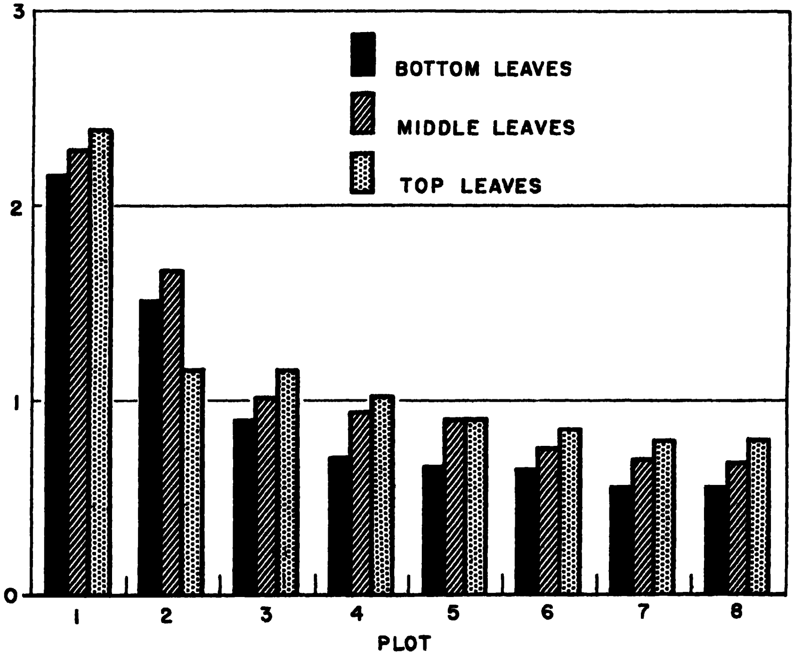

FIGURE 1.—The nitrogen/potassium ratio of bright leaf tobacco as influenced by fertilizer treatments, 1938 crop, best quality.

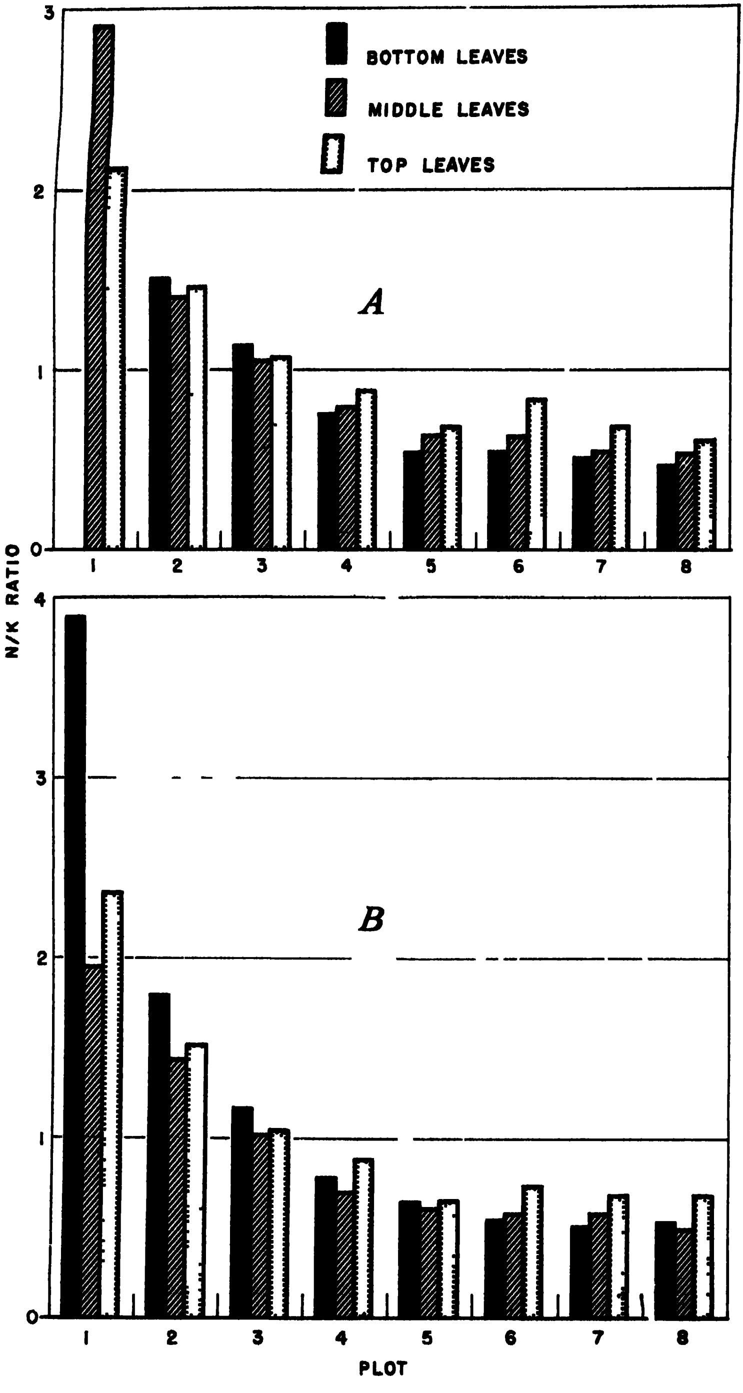

FIGURE 2.—The nitrogen-potassium ratio of bright leaf tobacco as influenced by fertilizer treatments, 1939 crop. *A*, Best quality; *B*, scrap quality.

TABLE 3.—*Effect of various fertilizer treatments on certain constituents of the bottom, middle, and top leaves of best-quality bright leaf cigarette tobacco produced on the experimental plots in 1938, and of best and scrap quality in 1939*

1938, BEST QUALITY

Plot No[1]	Location of leaves	Reducing materials calculated as glucose	Nitrogen	Potassium	Nitrogen/potassium ratio	Glucose/nitrogen ratio
		Percent	*Percent*	*Percent*		
1	Bottom	22 47	1 97	0 91	2 16	11 40
	Middle	13 48	2 60	4 14	2 28	5 18
	Top	5 87	3 22	1 35	2 38	1 83
2	Bottom	23 65	1 79	1 18	1 52	13 20
	Middle	20 06	2 11	1 26	1 67	9 50
	Top.	24 76	2 20	1 92	1 15	6 70
3	Bottom	24 03	1 71	1 91	90	14 05
	Middle	21 83	1 95	1 93	1 01	11 19
	Top	15 43	2 31	2 00	1 15	6 68
4	Bottom	27 94	1 59	2 20	72	17. 57
	Middle	22 18	2 07	2 17	95	10 72
	Top	18 60	2 15	2 11	1 02	8 65
5	Bottom	27 69	1 63	2 46	66	16 98
	Middle	24 45	2 09	2 29	91	11 68
	Top	14 18	2 35	2 59	91	6 03
6	Bottom	28 42	1 71	2 68	64	16 62
	Middle	22 63	1 86	2 44	76	12 17
	Top	13 35	2 32	2 71	85	5 75
7	Bottom	24 93	1 77	3 18	56	14 08
	Middle	20 89	1 98	2 81	70	10 55
	Top	18 62	2 21	2 78	79	8 44
8	Bottom	22 40	1 80	3 24	56	12 44
	Middle	25 29	1 85	2 72	68	13 68
	Top	18 19	2 20	2 74	80	8 27
1939, BEST QUALITY						
1	Bottom					
	Middle	14 00	2 12	0 73	2 91	6 61
	Top	6 54	2 74	1 29	2 12	2 39
2	Bottom	14 15	2 10	1 39	1 51	6 74
	Middle	17 21	2 05	1 46	1 40	8 40
	Top	12 46	2 20	1 51	1 46	5 66
3	Bottom	12 08	2 06	1 82	1 13	5 87
	Middle	17 75	1 84	1 75	1 05	9 65
	Top	9 68	2 31	2 15	1 07	4 19
4	Bottom	13 70	2 21	2 91	76	6 20
	Middle	22 58	1 93	2 43	79	11 71
	Top	11 02	2 27	2 57	88	4 86
5	Bottom	12 24	2 05	3 70	55	5 98
	Middle	20 09	1 91	3 05	63	10 51
	Top	8 93	2 36	3 48	68	3 78
6	Bottom	13 21	2 09	3 82	55	6 33
	Middle	21 50	1 89	2 90	63	11 37
	Top	13 60	2 21	2 65	83	6 15
7	Bottom	14 70	1 95	3 80	51	7 55
	Middle	20 62	1 92	3 50	55	10 73
	Top	10 17	2 33	3 38	69	4 36
8	Bottom	16 85	1 88	4 08	46	8 96
	Middle	20 97	1 86	3 44	54	11 30
	Top	9 59	2 21	3 54	62	4 34

TABLE 3. *Effect of various fertilizer treatments on certain constituents of the bottom, middle, and top leaves of best-quality bright leaf cigarette tobacco produced on the experimental plots in 1938, and of best and scrap quality in 1939*—Con.

1939, SCRAP QUALITY

Plot No.[1]	Location of leaves	Reducing materials calculated as glucose	Nitrogen	Potassium	Nitrogen/potassium ratio	Glucose/nitrogen ratio
		Percent	*Percent*	*Percent*		
1	Bottom	6.70	2.37	0.61	3.88	2.82
	Middle	12.48	2.13	1.09	1.96	5.86
	Top	3.70	3.12	1.32	2.36	1.19
2	Bottom	7.57	2.27	1.27	1.79	3.33
	Middle	17.22	1.93	1.35	1.43	8.93
	Top	9.12	2.41	1.61	1.52	3.71
3	Bottom	5.51	2.28	1.97	1.16	2.43
	Middle	14.39	1.92	1.89	1.02	7.50
	Top	7.49	2.51	2.44	1.04	2.95
4	Bottom	5.81	2.39	3.08	.78	2.43
	Middle	21.05	1.72	2.44	.71	12.25
	Top	9.86	2.23	2.55	.88	4.42
5	Bottom	7.83	2.16	3.33	.65	3.62
	Middle	16.31	1.98	3.17	.62	8.25
	Top	7.81	2.24	3.44	.65	3.49
6	Bottom	5.07	2.12	4.30	.56	2.09
	Middle	18.05	1.90	3.30	.58	9.51
	Top	9.46	2.41	3.22	.75	3.92
7	Bottom	7.72	2.34	4.53	.52	3.30
	Middle	16.74	1.96	3.31	.59	8.55
	Top	8.35	2.39	3.50	.68	3.49
8	Bottom	8.03	2.23	4.17	.54	3.60
	Middle	17.04	1.72	3.58	.48	9.92
	Top	7.08	2.36	3.48	.68	3.00

[1] See table 1 for formulas and quantities of fertilizers used on these plots.

DISCUSSION OF RESULTS

The results on the intake and accumulation of potassium as indicated in table 3 are somewhat in agreement with the observations of Anderson and coworkers (*1*), who stated that where the supply of potash in the soil was quite low, this element was found in higher concentrations in the upper rather than in the lower leaves. On the other hand, where the supply was ample, there was a higher accumulation in the lower leaves.

That the distribution of potassium in the plant is influenced in a measure by the quantities available is indicated by table 3. In plots 1 and 2 the low level of available potassium appeared insufficient to meet the demands of the growing plant. Under these conditions a larger accumulation of this element should be expected in the upper portions. However, where ample or rather excessive quantities of potassium were available to the plants, it appeared to be absorbed throughout the growth period, resulting in a marked accumulation of this element in the lower plant parts.

The incorporation of relatively large quantities of potassium in the fertilizer mixtures, improperly balanced with other necessary mineral nutrients, does not appear to be advisable, since the market value of the tobacco produced on plots so treated was found to be quite low. The conditions under which these plants were grown, however, indicate that quantities approximating 100 pounds per acre may be desirable.

While it is not advisable to produce bright leaf tobacco containing large quantities of nitrogen, this element must be given due consideration as supplying certain properties to the leaves which are extremely desirable from the standpoint of both yield and quality. From the results obtained, the writers are inclined to believe that the ratio of nitrogen to potassium should approximate 1:1 in the leaves. (See figs. 1 and 2.) It is apparent that the plots receiving minimum quantities of potassium had a wider ratio, while those receiving excessive quantities had a narrower ratio than this value. Leaves showing a ratio of approximately 1:1 were found on the middle leaves of the plants grown on plots 3, 4, and 5 during the 1938 season.

It would appear that there is some correlation between the nitrogen/potassium ratio of the leaves and the fertilizer treatment of the plants. While the 1938 and 1939 crops were produced under different weather conditions, the figures for the nitrogen/potassium ratio were of somewhat the same order.

Samples of the 1938 crop showed an increase in the concentration of nitrogen from the lower to the upper leaves. However, there appeared to be a slight variation where the potassium supply was more or less ample. On the other hand, the variation was quite pronounced for plot 1 where no potassium was applied. This was likewise noted for samples taken from this plot in 1939, while the remaining plots showed approximately the same concentration of nitrogen in the bottom and top leaves, being somewhat lower in the middle leaves.

It has been stated on numerous occasions that normal carbohydrate formation is closely associated with the presence of ample quantities of potassium (*9*, *8*, *5*). While the quantity of available soil potassium in plot 1 may have been sufficient to meet the needs of the plants during their early period of growth, it apparently was not sufficient to meet their demands during the latter stages of growth, as may be noted in the very low concentration of carbohydrates in the mature plants.

The results presented for the 1938 crop in table 3 are of particular interest. The samples from practically all plots showed a larger quantity of reducing materials in the lower than in the higher leaves. For those plots receiving sufficient potash this difference was less pronounced.

It is quite apparent that the reducing materials of the leaf are more or less associated with its quality. A comparison of the composition of the two grades of tobacco obtained from the 1939 crop, as given in table 3, is striking. In all cases, the reducing materials of the bottom and top leaves of the scrap quality was low, while that of the middle leaves was somewhat higher. It may be noted that while the bottom leaves of samples from plot 4 had approximately the same nitrogen/potassium ratio, the reducing materials of the scrap quality were less than half that of the best quality tobacco.

Harvesting of bright leaf tobacco is accomplished by the removal of two or three leaves at a time as they become "ripe," starting at the bottom and progressing upward. This procedure is called "priming" and in a normal season five or more primings are taken from each plant. It is obvious, therefore, that weather conditions prior to a priming may influence the composition of the leaves. The moisture content of the soil has a great deal to do with the absorption of mineral nutrients by plants and in this connection it should be remembered that above-normal rainfall was experienced in 1938 while rainfall was below normal in 1939.

SUMMARY AND CONCLUSIONS

Samples of bright leaf cigarette tobacco were obtained from each of eight experimental plots where potash was the only variable in the individual fertilizer treatments, ranging from none to 300 pounds per acre. Samples were taken from both the 1938 and 1939 crops and divided into three groups according to their position on the stalk, as bottom, middle, and top leaves. Only the best quality samples of the 1938 crop were analyzed, but in 1939 analyses were made of both scrap and best quality. From the results obtained it may be concluded:

(1) When the quantity of potassium available to the plants is low, a greater concentration of this element occurs in the upper leaves, and the content of reducing materials, including glucose, is quite low in all the leaves.

(2) When the quantity of potash at the disposal of the plants is satisfactory, there is a more or less uniform concentration of this element in the bottom, middle, and top leaves and the production of reducing materials attains a maximum. During the season of 1938 these conditions prevailed where potash was applied at the rate of 90 pounds per acre.

(3) Extremely large applications of potash give rise to an increased absorption of this element, with a larger accumulation in the bottom leaves. Under the conditions of these experiments, such applications of potash, improperly balanced in respect to other nutrient materials, failed to show any further favorable influence on the production of reducing materials than that observed in plants receiving adequate potash fertilization.

(4) Under the conditions of these experiments, a nitrogen/potassium ratio of from 0.8 to 1.1 appears to be highly desirable. Leaves of tobacco plants grown under these varied conditions showed a nitrogen/potassium ratio ranging from 0.5 to 3.9. Neither extreme was found to be associated with good quality.

Desirable nitrogen/potassium ratios were obtained in 1938 from applications of from 60 to 120 pounds of potash, while desirable nitrogen/potassium ratios were obtained in 1939 from 60 to 90 pounds of potash applied on the basis of 1 acre, which may be attributed in large measure to seasonal differences.

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USE OF SODIUM BIFLUORIDE AND SODIUM SILICOFLUORIDE IN THE DISINFECTION OF HIDES[1]

By C. A. Manthei, *assistant veterinarian*, and A. Eichhorn, *director, Animal Disease Station, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

The importation of hides and skins presents a hazard to the livestock industry of the United States because of the possible introduction of various disease germs or viruses that may adhere to or be incorporated in these materials. Such maladies as foot-and-mouth disease, rinderpest, and anthrax can be spread in this manner. Federal regulations covering the disinfection of hides and skins imported from countries in which such communicable diseases are known to exist, therefore, are rigidly enforced.

Research has been continuous in an effort to find some chemical substance or compound that would be effective in rendering all infected hides and skins safe for importation, without injuring them for tanning purposes. Various chemical substances have been found to be effective germicides or virucides when used with only the etiological agents of various diseases, but in the presence of animal tissue the disinfectants in many instances have been ineffective. This condition is due to the inability of some disinfectants to penetrate the tissues or to the formation of an insoluble substance by the combination of the chemical with the tissue proteins. Another equally important factor in hide disinfection is the effect of the disinfectants on the tanning properties of the hides. Some disinfectants have been found to be effective in destroying the contaminating germs or viruses, but they damaged the hides.

REVIEW OF LITERATURE

Most of the early work on hide disinfection was connected with anthrax. Extensive research was necessary to find methods of disinfection that would destroy both the anthrax bacilli and the spores. The most widely accepted methods were those recommended by Seymour-Jones (*11*)[2] and Schattenfroh (*10*). Work was done by Ponder (*9*), Smyth (*12*), Tilley (*13*), and O'Flaherty and Doherty (*7*) to determine the efficiency of these methods.

The prevention of foot-and-mouth disease in the United States is extremely important because of the enormous losses to livestock owners and the cost to the State and Federal Governments caused by the drastic methods of eradication. Much work has been done by various investigators toward the control of foot-and-mouth disease. The British Foot-and-Mouth Disease Research Committee of the Ministry of Agriculture and Fisheries, in its first (*2*), second (*3*), third (*4*), fourth (*5*), and fifth (*6*) progress reports, and the United States Foot-and-Mouth Disease Commission (*8*) found that agents which coagulate protein are ineffective against the virus in the presence of exudate or tissue. As a result, various noncoagulating protein chemicals were employed and found to be virucidal when used in the proper dilution.

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[1] Received for publication January 18, 1941.

[2] Numbers in parentheses refer to Literature Cited, p. 47.

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Among the chemicals mentioned are sodium hydroxide, sodium bisulfate, sodium bisulfite, antiformin, sulfoliquid, and formalin. Results obtained by Trautwein and Reppin (*14*), as well as Winkel (*15*), showed that 1 percent of sodium hydroxide killed the virus readily, and the latter worker used 0.1 to 2 percent of sulfuric acid with equal effectiveness. In the 1928 outbreak of foot-and-mouth disease in Bavaria, Germany, 1 percent of sodium hydroxide in conjunction with 5 percent of calcium hydroxide was successfully used to disinfect premises. Helm and Curtze (*1*) demonstrated that the efficiency of sulfoliquid and caustic soda was greatly increased when applied hot (100° C.).

In the third report of the British Foot-and-Mouth Disease Research Committee (*4*), mention is made of the use of formalin in the disinfection of hides heavily infected with foot-and-mouth-disease virus. In such hides soaked in 1-percent formalin solution for 48 hours, the virus was destroyed but the formalin had a detrimental effect on the hides. In the fourth report of that Committee (*5*), the statement is made that when infected hides were soaked in sodium bisulfate solution (1 to 10,000) for 5 hours or sodium bifluoride (1 to 20,000) for 2 hours, the virus was destroyed and no damage was done to the hides from the standpoint of leather production. No further mention was made of this method of hide treatment in subsequent publications nor were the protocols of the experiment given in that report.

To confirm this work, O'Flaherty and Doherty (*7*) used sodium bisulfate and sodium bifluoride in hides contaminated with the virus of vesicular stomatitis rather than foot-and-mouth disease, in accordance with the policy of the Bureau of Animal Industry of the United States Department of Agriculture not to experiment with the virus of foot-and-mouth disease in the United States. This has been a long-established policy because of danger to the livestock industry of the presence of foot-and-mouth disease virus in the country even for experimental purposes. These authors found that sodium bifluoride, in a solution of 1 to 10,000, destroyed the virus in 24 hours, whereas sodium bisulfate, in a solution of 1 to 400, failed to kill in 24 hours. No undesirable influence on the skin and subsequent leather was noticed by several tanneries that used the sodium bifluoride process of treatment.

PURPOSE AND EXPERIMENTAL PROCEDURE

To test the virucidal action of sodium bifluoride and sodium silicofluoride on hides impregnated with the virus of vesicular stomatitis, experiments were conducted in 1939 at the United States Department of Agriculture Animal Disease Station, Beltsville, Md. Virus of vesicular stomatitis was used because of its similarity to the virus of foot-and-mouth disease. The use of sodium bifluoride was suggested by the report of the British Foot-and-Mouth Disease Research Committee showing its value in destroying the virus of foot-and-mouth disease. Sodium silicofluoride was tested because it had been reported to be the most efficient of the fluorine compounds for curing or preserving hides.[3]

The vesicular virucidal virus was of the New Jersey type and was obtained from artificially infected cattle and horses. The lesions from these animals were dried, sealed in vacuo, and held at 4° C.

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[3] Test made at the suggestion of C E Senseman, Industrial Farm Products Research Division, Bureau of Agricultural Chemistry and Engineering, U. S Department of Agriculture.

This dried virus was inoculated on scarified metatarsal pads of guinea pigs. Only freshly harvested virus from guinea pigs with high temperatures at 24 to 28 hours and good lesions at 48 hours after inoculation was used in the experiments. The use of a virus after only a single passage through guinea pigs eliminated as much as possible any changes in virulence or structure of the virus that might occur from continued passage in laboratory animals or live-tissue culture.

The tests included two methods of determining the virucidal action of the disinfectants: Use of (1) infected guinea pig pads placed with calfskin in the various soaks, and (2) calfskin artificially impregnated with the virus and placed in the various soaks. In each method, soak solutions were prepared of sodium bifluoride and sodium silicofluoride. Untreated tap water from a well was used as a control. The tests were repeated twice and identical results were obtained unless otherwise stated. The entire experiment was carried out at room temperature (20° to 24° C.).

In the first method, sodium bifluoride and sodium silicofluoride were dissolved in tap water in dilutions of 1 to 5,000, 1 to 10,000, and 1 to 20,000. The three dilutions for each of the two chemicals were placed in hard rubber vats, which were used instead of glass jars to eliminate any possibility of chemical reaction between the fluorine and the elements in glass. During the soaking periods, the vats were covered to prevent the admittance of light, thus eliminating any possible harmful effect on the virus. Hydrogen-ion determinations were made on each dilution before soaking and after 24 and 48 hours of soaking. Infected metatarsal pads from 6 guinea pigs and 10 pieces weighing 50 gm. each of salt-cured calfskin were placed in 2,500 gm. of each dilution of test solution and held at room temperature for 24- and 48-hour periods. At the end of each holding period, half of the guinea pig pads were removed from each dilution of test solution, washed in 0.85-percent saline solution, and ground in a sterile mortar. Then an 0.85-percent saline solution was added in sufficient quantity to make a 10-percent suspension. These suspensions were shaken thoroughly and then allowed to settle. The supernatant fluid from each suspension was inoculated on scarified metatarsal pads of guinea pigs; all pads were covered with sterile gauze for 24 hours. Temperatures were taken on the guinea pigs at 24 to 28 hours after inoculation, and the metatarsal pads were examined for lesions after 48 hours. These guinea pigs were saved for 13 to 21 days and then reinoculated with fresh virus to determine whether any immunity had been acquired as well as to eliminate the possibility of false clinical manifestations that may have been caused by the test solutions. In addition to the control experiments involving the placing of affected guinea pig pads in tap water, control experiments were also carried out with normal guinea pigs to determine the virulence of the virus used in all the soak solutions as well as that used to test the animals for immunity.

In the second method, conditions were the same as in the first except that 1 gm. of infected pads from guinea pigs was ground in a mortar with 5 cc. of 0.85-percent saline solution, and 0.25 cc. of this suspension of virus was then injected intradermically into circular pieces of calfskin, each 1 inch in diameter and weighing approximately 1 gm. Six inoculated pieces were placed between 50-gm. pieces of calfskin in each of the dilutions of sodium bifluoride and sodium silicofluoride. The proportion of the 50-gm. pieces of skin to soak solution was 1 to 5 by weight as in the first method. Half of the impregnated circular pieces of calfskin were removed from the test solutions at 24 hours and the remaining half at 48 hours. In each instance they were washed in 0.85-percent saline solution, cut into very small pieces with sterile scissors, placed in a mortar, and ground to a pulpy consistency. This material was inoculated on guinea pig pads, the remainder of the procedure being the same as described in the first method. The purpose of this type of experiment was to determine the penetrating ability of the soak solution and to duplicate naturally infected skins as nearly as possible.

RESULTS

The results obtained by the first method are shown in table 1 The sodium bifluoride, in the three dilutions used, destroyed the virus of vesicular stomatitis in the guinea pig pads after 24 hours of soaking. Sodium silicofluoride, in dilutions of 1 to 10,000 and 1 to 5,000, destroyed the virus in 24 hours. The 1 to 20,000 dilution killed the virus only after 48 hours of soaking. The virus was still viable after 48 hours of soaking in tap water at room temperature Results with the normal control guinea pigs demonstrated the virulence of the virus used in all the soak solutions as well as that used to test guinea pigs for immunity.

TABLE 1 *Effect of sodium bifluoride and sodium silicofluoride on virus of vesicular stomatitis in guinea pig pads, in the presence of salt-cured calfskins, 1939*

Disinfectant		Length of soak	Guinea pig No	Guinea pig data[1] on						
				Test of virulence				Test of immunity[2]		
Kind	Dilution			Date of inoculation	Temperature at 24 hours	Lesions at 48 hours on		Temperature at 24 hours	Lesions at 48 hours on	
						Left pad	Right pad		Left pad	Right pad
		Hours			°F			°F		
Sodium bifluoride	1 5 000	24	664	Oct 10	101.8	o	o	104.6	d	d
	1 10 000		670		101.7	o	o	105.1	d	d
	1 20 000		667		103.2	o	o	105.0	d	d
	1 5,000	48	825	Oct 11	103.2	o	o	105.4	c	d
	1 10 000		816		101.6	o	o	105.6	d	d
	1 20 000		818		102.2	o	o	105.9	d	d
Sodium silicofluoride[3]	1 5 000	24	663	Oct 10	102.5	a	o	105.6	d	d
	1 10 000		699		101.8	o	o	105.3	d	d
	1 20 000		674		102.9	a	o	104.6	b	d
	1 5 000	48	822	Oct 11	102.0	o	o	105.3	d	d
	1 10 000		801		102.4	o	o	105.1	d	d
	1 20 000		815		103.0	o	o	106.1	d	d
Tap water (controls)[4]		24	1078	Oct 10	105.2	d	d	101.6	o	o
			1087		105.1	d	d	102.4	o	o
			1092		105.0	d	d	102.1	o	o
			1098		105.8	d	d	101.5	o	o
		48	1081	Oct 11	106.0	d	d	103.0	o	o
			1089		106.0	d	d	102.1	o	o
			1090		105.8	d	d	102.5	o	o
			1093		106.4	d	d	102.6	o	o
None (normal controls)			1	Oct 9	105.4	d	d			
			2		105.1	d	d			
			3		105.6	d	d			
			4		106.0	d	d			
			5					106.2	d	d
			6					105.0	d	d
			7					105.2	d	d
			8					105.7	d	d

[1] Key o, none, a, slight (swelling and tenderness), b moderate (swelling and tenderness plus a few small vesicles along the hair line) c, good (pad loosened tenderness plus incomplete vesicle formation around and under entire pad), d, severe (pad loosened, complete vesicle formation under and around pad and sloughing)

[2] Fresh virus of vesicular stomatitis from guinea pig pads was used date of inoculation in all instances, Oct 28, 1939

[3] 12 affected guinea pig pads used in each dilution of the soak solution

[4] 12 affected guinea pig pads used

The results obtained by the second method are shown in table 2. With sodium bifluoride, in dilutions of 1 to 10,000 and 1 to 5,000, the virus was destroyed after 24 hours of soaking. In the 1 to 20,000 dilution, the virus was destroyed in 50 percent of the cases in 24 hours, but the destruction of virus was complete only after 48 hours of soaking.

With sodium silicofluoride, 1 to 10,000 and lower dilutions destroyed the virus in 24 hours, whereas the 1 to 20,000 dilution completely destroyed the virus in only 50 percent of the cases after 48 hours of soaking.

TABLE 2.—*Effect of sodium bifluoride and sodium silicofluoride on virus of vesicular stomatitis inoculated into salt-cured calfskins, 1939*

Disinfectant			Guinea pig data							
					Test of virulence			Test of immunity		
Kind	Dilution	Length of soak	Guinea pig No.	Date of inoculation	Temperature at 24 hours	Lesions at 48 hours on Left pad	Lesions at 48 hours on Right pad	Temperature at 24 hours	Lesions at 48 hours on Left pad	Lesions at 48 hours on Right pad
		Hours			°F.			°F.		
Sodium bifluoride	1:5,000	24	975	Oct. 18	102.0	o	o	101.1	d	d
	1:5,000		755		102.[illegible]	o	o	105.1	d	d
	1:10,000		971		102.9	o	o	105.4	d	d
	1:10,000		8[illegible]1		103.0	o	o	104.8	d	d
	1:20,000		8[illegible]8		103.1	o	o	107.0	d	d
	1:20,000		961		103.[illegible]	b	a	103.0	a	a
	1:5,000	48	948	Oct. 19	102.8	o	o	104.1	d	d
	1:5,000		932		102.7	o	o	105.[illegible]	c	c
	1:10,000		[illegible]68		102.0	o	o	105.3	d	d
	1:10,000		788		102.0	o	o	104.8	d	d
	1:20,000		970		102.0	o	o	105.1	d	d
	1:20,000		928		103.0	o	o	105.0	d	d
Sodium silicofluoride	1:[illegible],000	24	86[illegible]	Oct. 18	102.1	o	o	101.7	d	d
	1:5,000		867		103.1	o	o	104.4	d	d
	1:10,000		8[illegible]1		103.0	o	o	103.8	c	c
	1:10,000		866		102.6	o	o	104.6	d	d
	1:20,000		8[illegible]1		102.8	o	a	101.6	d	c
	1:20,000		870		103.3	a	o	102.6	b	b
	1:[illegible],000	48	936	Oct. 19	101.7	o	o	104.[illegible]	d	d
	1:5,000		937		101.9	o	o	10[illegible].8	d	d
	1:10,000		942		103.0	o	o	105.3	d	d
	1:10,000		94[illegible]		102.5	o	o	104.8	d	d
	1:20,000		927		103.0	o	a	103.6	b	a
	1:20,000		949		102.8	o	o	104.6	d	d
Tap water (controls)		24	1082	Oct. 18	10[illegible].2	d	d	101.8	o	o
			913		105.5	d	d	102.4	o	o
			1097		101.8	d	d	102.2	o	o
			1069		106.2	d	d	101.4	o	o
		48	1001	Oct. 19	104	d	d	102.0	o	o
			1071		105.6	d	d	101.8	o	o
			1076		105.0	d	d	102.6	o	o
			1080		104.8	d	d	102.2	o	o
None (normal controls)			9	Oct. 17	105.2	d	d			
			10		10[illegible].0	d	d			
			11		105.6	d	d			
			12		10[illegible].4	c	d			
			95[illegible]					105.4	d	d
			910					101.9	d	d
			[illegible]5					105.4	d	d
			777					101.[illegible]	d	d

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[1] Key: o, none; a, slight (swelling and tenderness); b, moderate (swelling and tenderness plus a few small vesicles along the hair line); c, good (pad loosened, tenderness plus incomplete vesicle formation around and under entire pad); d, severe (pad loosened, complete vesicle formation under and around pad and sloughing).

[2] Fresh virus of vesicular stomatitis from guinea pig pads was used; date of inoculation in all instances, Oct. 31, 1939.

[3] 6 1-gm. pieces of calfskin, each injected with 0.25 cc. of affected guinea pig pad emulsion, used in each dilution of soak solution.

[4] 6 1-gm. pieces of calfskin, each injected with 0.25 cc. of affected guinea pig pad emulsion, used

The virus was still viable after 48 hours of soaking in tap water. Similar results were obtained with the control guinea pigs as in the first method.

The hydrogen-ion concentration of tap water and the various solutions is shown in table 3. All the soaks tended to be less acid with increase in length of time, but none of the solutions reached alkalinity. However, the virucidal action of these solutions was not comparable with the degree of acidity as sodium silicofluoride in the comparative dilutions had in almost every instance a higher acidity than sodium bifluoride, whereas sodium bifluoride, in the virulence tests, was the more virucidal.

TABLE 3.—*Average hydrogen-ion concentration of the soak solutions at various periods in all experiments*

Soak solution	pH value		
	Before soaking	After 24 hours	After 48 hours
Sodium bifluoride			
1-5,000	3.7	4.7	5.4
1-10,000	3.75	5.6	5.9
1-20,000	3.8	6.0	6.2
Sodium silicofluoride			
1-5,000	3.5	4.6	5.0
1-10,000	3.55	5.2	5.7
1-20,000	3.6	5.7	6.2
Tap water	6.6	6.85	7.2

DISCUSSION

Results obtained from the studies indicate that both sodium bifluoride and sodium silicofluoride are virucidal to the virus of vesicular stomatitis. Sodium bifluoride, in a dilution of 1 to 20,000, was slightly more effective than sodium silicofluoride under like conditions.

A longer time was required for the higher dilutions of the disinfectants to kill the virus injected intradermically in hides than to kill the virus in the guinea pig pads. This fact was due, no doubt, to the extra time required by the disinfectants to penetrate the skin before coming in contact with the virus. After the skins had been in the various soak solutions for 24 to 48 hours, a greater thickness was noted in the skins soaked in the two disinfectant solutions than in those soaked in tap water.

It appears that the sodium chloride in the cured skins had little influence on the effectiveness of sodium bifluoride or sodium silicofluoride, as its presence on the skins in tap water failed to affect the virulence of the virus.

The importance of sodium bifluoride and sodium silicofluoride for use in hide and skin disinfection depends on their effectiveness against foot-and-mouth disease virus. The present experiments showed that these disinfectants destroyed vesicular stomatitis virus after 24 hours of soaking in dilutions of 1 to 10,000. The British Foot-and-Mouth Disease Research Committee in its fourth report (*5*) states that a dilution of 1 to 20,000 of sodium bifluoride killed foot-and-mouth disease virus in 2 hours, and O'Flaherty and Doherty (*7*) found that a dilution of 1 to 10,000 of sodium bifluoride destroyed vesicular stomatitis virus after 24 hours.

From these data it may be assumed that the use of either sodium bifluoride or sodium silicofluoride, in a solution of 1 to 10,000 for 24 hours at room temperature, would be effective in the disinfection of hides or skins infected with foot-and-mouth disease when the ratio of hide or skin to soak solution is 1 to 5 by weight.

SUMMARY AND CONCLUSION

The efficiency of sodium bifluoride and sodium silicofluoride as hide disinfectants was studied, the virus of vesicular stomatitis being used as the contaminant. This work was carried on at the United States Department of Agriculture Animal Disease Station, Beltsville, Md., in 1939.

Two methods of approach were used: (1) Infected guinea-pig pads soaked for 24 and 48 hours in solutions of 1 to 5,000, 1 to 10,000, and 1 to 20,000 of the two fluorine compounds, in the presence of salt-cured calfskins; (2) sections of salt-cured calfskin injected intradermically with vesicular stomatitis virus in the aforementioned soak solutions. In both types of experiments, tap water was used as a control. The proportion of salt-cured skin to the quantity of soak solution in all experiments was 1 to 5 by weight. All experiments were conducted at room temperature.

The results were as follows:

In the first experiment, sodium bifluoride killed the virus in guinea-pig pads in all three dilutions in 24 hours. Sodium silicofluoride killed the virus in dilutions of 1 to 5,000 and 1 to 10,000 in 24 hours and in all dilutions in 48 hours

In the second experiment, sodium bifluoride killed the virus in artificially inoculated calfskins in dilutions of 1 to 5,000 and 1 to 10,000 in 24 hours and in all dilutions in 48 hours. Sodium silicofluoride killed the virus in dilutions of 1 to 5,000 and 1 to 10,000 in 24 hours but was not completely effective in the dilution of 1 to 20,000 in 48 hours.

Tap water did not affect the virus in either guinea-pig pads or calfskins after 24 or 48 hours of soaking.

The hydrogen-ion concentration of the various soak solutions was determined at the beginning and after 24 and 48 hours of soaking. Sodium silicofluoride, at equal dilutions, had a higher hydrogen-ion concentration than sodium bifluoride, both decreasing with length of time. However, a high hydrogen-ion concentration does not necessarily indicate greater virucidal powers The hydrogen-ion concentration of tap water also decreased with length of time.

By analogy with similar research by the British Foot-and-Mouth Disease Research Committee, it is a logical assumption that sodium bifluoride and sodium silicofluoride are also effective in destroying the virus of foot-and-mouth disease.

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THE EFFECT OF FERTILIZATION AND CULTURAL PRACTICES ON THE OIL AND AMMONIA CONTENT OF COTTONSEED GROWN ON YAZOO-MISSISSIPPI DELTA SOILS[1]

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INTRODUCTION

The cotton-oil mills of the Yazoo-Mississippi Delta usually pay a premium on cottonseed grown in that area. Since this is one of the world's greatest cotton producing areas, it becomes a question of considerable importance to both the producer and processor to know what factors are responsible for this quality product so that greater use may be made of this apparent natural advantage in the highly competitive field of cotton production.

The market value of cottonseed is determined by the percentages of oil, meal, hulls, and linters which the seeds contain. Pound for pound, the oil is the most valuable constituent of the seed, but since the percentage of meal is roughly three times that of the oil, the meal represents the higher total money value per acre. Garner et al.[3] have shown that cottonseed produced on different types of soil may differ somewhat in its content of oil and ammonia. Certain oil-mill records[4] indicate that cottonseed produced on the alluvial soils of the Yazoo-Mississippi Delta, which are among the most productive soils in the Cotton Belt, may contain a higher percentage of both oil and ammonia than cottonseed produced in the upland or less fertile parts of Mississippi.

Cultural practices in cotton production are not uniform, even in the same section, and any relationship that might be established between soil fertility and the composition of cottonseed would not have its widest application without a knowledge of the effect, if any, produced by different cultural practices. To determine to what extent these variations in cultural methods might affect the oil and ammonia content of the cottonseed, a study of the effect of the cultural pactices most commonly followed in the Yazoo-Mississippi Delta was included in this investigation.

METHODS AND MATERIALS

Samples of cottonseed were collected from $\frac{1}{60}$-acre field plots, replicated five to nine times, at the Delta Branch Experiment Station, Stoneville, Miss., and analyzed for oil, ammonia, and moisture content in accordance with the methods of the National Cottonseed Products Association. The data are reported on a 10-percent moisture basis.

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[2] The valuable assistance of Dr. Rowland Cowart of this station in the preparation of the manuscript is gratefully acknowledged

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[4] Unpublished records of the Tallahatchie Oil Mill, Webb, Miss

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The plots from which the samples were taken had received annually uniform treatments for a period of 10 years prior to the first sampling. The sampling was made annually from 1933 to 1937, inclusive.

The fertility treatments or cultural practices may be grouped as follows: (1) Commercial fertilizers, which supplied nitrogen from different sources and at different rates, phosphorus from superphosphate, and potassium from sulfate of potash at the rate of 45 pounds P_2O_5 and 37½ pounds K_2O per acre, respectively; (2) green manures, which included hairy vetch, Austrian winter peas, sweetclover, burclover, and rye; and (3) cultural practices, which included (a) Variations in methods of seedbed preparation consisting of no plowing, bedding in fall, bedding in spring, and bedding in fall and rebedding in spring, all followed by uniform cultivation; (b) uniform seedbed preparation, followed by weekly cultivations for each depth, which included hoeing only, harrowing, cultivating 3, 6, and 6 inches deep followed by 3 inches deep; and (c) variations in spacing of plants within the hills, the number of plants per hill ranging from two to seven with a uniform distance of 16 by 40 inches between hills.

EXPERIMENTAL RESULTS

COMMERCIAL FERTILIZERS

NITROGEN

The data in table 1 show the effect which 30 pounds of nitrogen per acre applied in the form of various commercial nitrogenous fertilizers had on the oil and ammonia content of cottonseed grown on Sarpy loam soil. There seems to have been no effect on the oil content of the cottonseed regardless of the source of nitrogen, but the ammonia content was increased considerably by nitrogen from all of the different sources, though least by that from organic sources.

TABLE 1.—*Effect of nitrogen from different sources on the yield and on the oil and ammonia content[1] of cottonseed grown on Sarpy loam*

Source of nitrogen (30 pounds per acre)	Yield of cottonseed per acre	Oil content	Ammonia content
	Pounds	*Percent*	*Percent*
No nitrogen	616	20.88±0.10	3.41±0.03
Sodium nitrate	980	20.19± .25	3.71± .07
Ammonium nitrate	935	20.37± .20	3.66± .09
Ammonium sulfate	916	21.05± .18	3.66± .07
Cyanamid	914	20.36± .19	3.62± .06
Cottonseed meal	975	20.51± .15	3.53± .13

[1] Standard error is used to indicate the degree of significance.

Table 2 gives the data on the performance of nitrogen from two sources, sodium nitrate and cyanamid, applied at rates ranging from 7½ to 45 pounds of nitrogen per acre at 7½-pound intervals. Nitrate of soda is immediately soluble, but cyanamid has to undergo certain chemical changes in the soil before the nitrogen is available to the plants. However, the general trend in the behavior of the two types of nitrogen carriers at the different rates of application was essentially the same. At the higher rates the oil percentages were lower for both sources, but the yield of oil per acre was greater since with the higher rates of fertilization a greater quantity of cottonseed was produced per acre. The percentage of ammonia in the cottonseed increased with the higher rates of application of nitrogen. Since the higher rates of nitrogen produced more pounds of cottonseed per acre, the higher rates likewise increased the total amount of ammonia to a considerable extent. While some of the increase in percentage of ammonia and the decrease in percentage of oil at the lower rates of nitrogen are not statistically significant, a trend is exhibited in both cases.

TABLE 2.—*Effect of applying at different rates nitrogen derived from sodium nitrate and cyanamid on the yield and on the oil and ammonia content* [1] *of cottonseed grown on Sarpy loam*

Nitrogen applied per acre (pounds)	Results with sodium nitrate: Yield of cottonseed per acre	Results with sodium nitrate: Oil content	Results with sodium nitrate: Ammonia content	Results with cyanamid: Yield of cottonseed per acre	Results with cyanamid: Oil content	Results with cyanamid: Ammonia content
	Pounds	*Percent*	*Percent*	*Pounds*	*Percent*	*Percent*
0	579	20.87±0.15	3.44±0.11	1,030	19.48±0.26	3.97±0.06
7½	656	20.83± .10	3.43± .03	1,052	19.47± .15	4.00± .03
15	724	20.58± .10	3.53± .03	1,066	19.54± .14	3.95± .06
22½	804	20.58± .13	3.63± .05	1,066	19.44± .12	4.06± .02
30	887	20.12± .21	3.71± .04	1,112	19.29± .13	4.07± .02
37½	946	19.89± .28	3.78± .05	1,113	19.13± .12	4.24± .08
45	987	19.81± .33	3.83± .05	1,150	19.01± .12	4.11± .06

[1] See footnote 1, table 1.

TABLE 3.—*Effect of phosphorus and potassium, when nitrogen was supplied by sodium nitrate and cyanamid, on the yield and on the oil and ammonia content* [1] *of cottonseed grown on Sarpy loam*

Treatment	Results with sodium nitrate (30 pounds of nitrogen per acre): Yield of cottonseed per acre	Results with sodium nitrate (30 pounds of nitrogen per acre): Oil content	Results with sodium nitrate (30 pounds of nitrogen per acre): Ammonia content	Results with cyanamid (30 pounds of nitrogen per acre): Yield of cottonseed per acre	Results with cyanamid (30 pounds of nitrogen per acre): Oil content	Results with cyanamid (30 pounds of nitrogen per acre): Ammonia content
	Pounds	*Percent*	*Percent*	*Pounds*	*Percent*	*Percent*
None	1,028	19.47±0.30	3.92±0.05	963	19.49±0.18	4.17±0.05
Phosphorus	1,092	19.70± .14	3.86± .06	935	19.20± .13	4.21± .02
Phosphorus and potassium	1,059	20.10± .14	3.94± .07	983	19.60± .23	4.13± .03
Potassium	1,059	19.76± .13	3.92± .04	983	19.33± .21	4.09± .07

[1] See footnote 1, table 1.
[2] 45 pounds per acre of P_2O_5.
[3] 37½ pounds per acre of K_2O.

PHOSPHORUS AND POTASSIUM

Table 3 shows the results from adding phosphorus and potassium with applications of sodium nitrate and cyanamid to make mixed fertilizers carrying 45 pounds of P_2O_5, 37½ pounds of K_2O, and 30 pounds of nitrogen per acre. The ammonia content of the seed was not appreciably affected in any case. It will be observed also that when phosphorus or potassium was used either alone or in combination, the effect on the oil content of the cottonseed was not statistically significant. These results are in agreement with those of Garner, Allard, and Foubert,[5] who reported that nitrogen affects the oil and ammonia content of cottonseed but that phosphorus and potassium do not. They are not, however, in full agreement with the results of O'Kelly, Hull, and Gieger,[6] who found that the fertilization of cotton with potash raised the oil content but lowered the protein content of the seed. This difference in results may be due in part at least to a difference in the natural supply of potash in the soils used in the two investigations. The work of O'Kelly et al. was carried out on soils so deficient in phosphorus and potassium that cotton responded well to phosphate and potash fertilization, whereas the present work was conducted on a soil that ordinarily has not given economical increases in cotton yield from either phosphate or potash fertilization. It is known that a soil extremely deficient in an element will give a greater response in yield to applications of that element than will soils only moderately deficient in it.

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[5] See footnote 3.

GREEN MANURES

The availability of the nutrients supplied in green manures and the effect of organic matter on soil moisture are two factors that may cause the plant nutrients supplied through commercial fertilizers and green manures to affect the oil and ammonia content of the cottonseed differently. To reduce this source of variability, a number of green manure crops were included in the test, namely, hairy vetch, Austrian winter peas, sweetclover, bur-clover, and rye. Table 4 gives the data on the effect of turning under green manures on the oil and ammonia content of cottonseed. Since some of the crops made more growth than others, different quantities of nitrogen were turned under in the different legumes, a condition which is beyond control in field experimentation. The results with the legumes are similar to those obtained with the commercial sources of nitrogen, that is, the oil percentage was decreased and the ammonia percentage increased by the addition of nitrogenous manure. The results with rye, which is a nonleguminous crop, differed from those of the nitrogenous fertilizers but were very similar to those obtained on the no-treatment plot

CULTURAL PRACTICES

Since the method of preparing the seedbed, the frequency and depth of cultivation, and the number of plants grown per hill, are practices that vary widely, not only between different cotton-growing regions, but within a single community, an attempt was made to include a wide range of these practices in the investigations. The effect on yield and on oil and ammonia content of the cottonseed resulting from these variations in cultural practices are shown in tables 5, 6, and 7. It will be observed that none of the variations in seedbed preparation, cultivation, or spacing seem to have had any marked effect on the oil or ammonia content of the seed. Any outstanding advantage of one of these practices over another in the growing of cotton would have to be determined on the basis of quantity production and economics rather than on the oil and ammonia content of the seed produced.

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[6] O'KELLY, J. F., HULL, W. W., and GIEGER, M. EFFECTS OF VARYING AMOUNTS OF POTASH ON OIL AND PROTEIN AND ON THE WEIGHT AND PERCENTAGE OF COTTONSEED. Miss. Agr. Expt. Sta. Tech. Bul. 20, 8 pp., illus. 1933.

TABLE 4.—*Effect of green manures on the yield and on the oil and ammonia content*[1] *of cottonseed grown on Sarpy loam*

Green manure used	Nitrogen supplied	Yield of cottonseed per acre	Oil content	Ammonia content
	Pounds	*Pounds*	*Percent*	*Percent*
None		712	21.20±0.17	3.43±0.07
Austrian winter peas	75	1,071	19.64± .16	3.67± .07
Hairy vetch	85	1,16[illegible]	19.35± .16	3.68± .06
Sweetclover	58	940	19.89± .22	3.48± .07
Bur clover	114	931	19.77± .30	3.59± .09
Rye		603	20.43± .22	3.23± .07

[1] See footnote 1, table 1.

TABLE 5.—*Effect of varying the method of seedbed preparation on the yield and on the oil and ammonia content*[1] *of cottonseed grown on Sarpy loam and on Sharkey clay soil*

Method of seedbed preparation	Results on Sarpy loam soil			Results on Sharkey clay soil		
	Yield of cottonseed per acre	Oil content	Ammonia content	Yield of cottonseed per acre	Oil content	Ammonia content
	Pounds	*Percent*	*Percent*	*Pounds*	*Percent*	*Percent*
None, stalks pulled	531	19.11±0.37	3.85±0.05	571	19.33±0.23	3.91±0.05
Bedded in fall	708	19.53± .33	3.71± .04	570	19.34± .27	3.89± .06
Bedded in spring	808	19.09± .18	3.83± .0[illegible]	[illegible]97	19.51± .16	3.95± .0[illegible]
Bedded in fall and rebedded in spring	874	19.03± .17	3.82± .04	717	19.47± .15	3.80± .0[illegible]

[1] See footnote 1, table 1.

TABLE 6.—*Effect of varying the method, frequency, and depth of cultivation on the yield and on the oil and ammonia content*[1] *of cottonseed grown on Sarpy loam*

Kind of cultivation	Interval of cultivation	Yield of cottonseed per acre	Oil content	Ammonia content
		Pounds	*Percent*	*Percent*
None, hoe only	Weekly	923	19.43±0.2[illegible]	3.92±0.05
Harrow 1 inch deep or less	do	914	18.87± .21	3.67± .06
Plow 3 inches deep	Semiweekly	1,050	18.98± .32	3.91± .06
Do	Weekly	1,039	18.78± .10	3.98± .05
Plow 6 inches deep	do	919	18.90± .20	3.[illegible]± .03
Plow 6 inches deep (3 times at weekly intervals) and plow 3 inches deep afterwards	do	1,003	19.28± .12	3.92± .06

[1] See footnote 1, table 1.

TABLE 7.—*Effect of number of plants per hill on yield and on oil and ammonia content*[1] *of cottonseed grown on Sarpy loam*

[Hills spaced 16 by 40 inches]

Plants per hill (number)	Yield of cottonseed per acre	Oil content	Ammonia content
	Pounds	*Percent*	*Percent*
2	69[illegible]	19.28±0.08	3.85±0.09
3	6[illegible]8	19.40± .17	3.83± .0[illegible]
4	721	19.53± .14	3.86± .0[illegible]
[illegible]	737	19.[illegible]1± .19	3.85± .05
6	719	19.41± .24	3.92± .04
7	711	19.1[illegible]± .17	3.96± .09

[1] See footnote 1, table 1.

SUMMARY

Samples of cottonseed were collected annually over a 5-year period from 1933 to 1937, inclusive, on plots of Sarpy loam located at the Delta Branch Experiment Station, Stoneville, Miss., which had received for a 10-year period prior to the first sampling the following treatments: (1) Commercial fertilizers, which included nitrogen, phosphorus, and potash; (2) green manures, which included hairy vetch Austrian winter peas, sweetclover, bur clover, and rye; and (3) a variety of cultural practices, which included (*a*) different methods of seedbed preparation, namely, no plowing, bedding in the fall, bedding in the spring, bedding in the fall and rebedding in the spring; (*b*) uniform seedbed preparation followed by different methods of cultivation, which included hoeing only, harrowing only, cultivating 3 inches deep, 6 inches deep, 6 inches deep followed by 3 inches deep, and (*c*) variations in number of plants per hill with uniform spacing between hills

The percentage of oil and ammonia was determined on all samples of cottonseed, with the following results: (1) Nitrogenous fertilizers decreased the percentage of oil in the seed but increased the percentage of ammonia; (2) phosphorus and potassium when used separately gave no increase in oil percentage, but when used together gave a slight increase, although its significance may be questioned; the percentage of ammonia was unaffected in either case; (3) green manures, like commercial fertilizers, increased the percentage of ammonia and decreased the percentage of oil on the basis of their nitrogen content.

The different methods used in preparing the seedbed, cultivating and spacing showed little if any influence on the percentage of oil and ammonia in the cottonseed. The quantity of oil and ammonia produced per acre was influenced somewhat by the different cultural practices, but any advantage of one practice over another is better measured by the quantity of oil and ammonia produced on the basis of acre yield than by the percentage of oil and ammonia in the cottonseed. Nitrogen in whatever form applied increased the ammonia content of the cottonseed.

FURTHER STUDIES OF THE PHOTOPERIODIC BEHAVIOR OF SOME MINTS (LABIATAE)[1]

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INTRODUCTION

The following paper is a partial report of studies of some of the members of the Labiatae begun long ago, the results of which, in part, have been reported in a recent paper.[2]

In the present paper a report is made upon a number of well-known commercial species grown in the United States for their valuable aromatic oils. It seems particularly desirable to learn as much as possible of the ecological requirements of these commercial species, some of which are rather extensively cultivated in certain regions of the United States at the present time.

Since cultivation of some of these mints may be extended and problems of crossing that are being investigated require a timely synchronization in order to secure flowers and pollination of certain species that do not normally have the same flowering season, the following data may be of some interest to those engaged in such work.

MATERIAL AND METHODS

The following species and strains were used in the work herein reported:[3] *Monarda didyma* L., *M. punctata* L., *Mentha arvensis* L., *M. citrata* Ehrh., *M. piperita* L., *M. spicata* L. (*M. viridis* L.), and *M. piperita* var.[4]

Clumps of each of these plants were taken from material growing in the field at Arlington, Va., on May 1, when the plants had just started to produce new growth. On this date good-sized clumps were transplanted to large, galvanized-iron buckets of 14-quart capacity, and the tests were begun May 2, at the Arlington Experiment Farm, Arlington, Va., at approximately lat. 39° N. and with a maximum length of day of 14.9 hours on June 21.

Lengths of day of 10, 12, 12½, 13, 13½, 14, 14½ hours, full day length, and 18 hours were afforded the plants. For all plants, except those receiving 18 hours of illumination daily, natural daylight was used. For photoperiods of 10 to 14½ hours inclusive, the natural daylight period was shortened by means of ventilated lightproof houses into which the plants were run on a rigid schedule each day to obtain the required number of hours of daily exposure. For this purpose large ventilated houses were used, equipped with tracks upon which large movable trucks bearing the containers were mounted.

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[1] Received for publication May 2, 1941.

[2] ALLARD, H. A., and GARNER, W. W. FURTHER OBSERVATIONS ON THE RESPONSE OF VARIOUS SPECIES OF PLANTS TO LENGTH OF DAY. U. S. Dept. Agr. Tech. Bul. 727, 64 pp., illus. 1940. (See pp. 49-55.)

[3] Except where otherwise noted, the material reported upon was kindly supplied by the Division of Drug and Related Plants, Bureau of Plant Industry.

[4] This unidentified variety of *M. piperita* was obtained from Vick Chemical Co., Greensboro, N. C.

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Before the completion of the experiments, the longer daily light periods were terminated by the natural seasonal shortening of the days. After this time the plants experienced the normal length of day with its daily decrements until the tests were discontinued. On the basis of the periods between actual sunrise and sunset, the 14½-hour day was terminated by the seasonal shortening on July 23; the 14-hour day was terminated on August 8; the 13½-hour day, on August 21; the 13-hour day, on September 3; the 12½-hour day, on September 15; and the 12-hour day, on September 27. However, before and after sunset there is some effective light that may extend the length of day perhaps ½ to ¾ of an hour. If this increase amounted to ¾ of an hour each day, the 14½-hour day would not terminate until August 13, and the 14-, 13½-, 13-, 12½- and 12-hour days would extend to August 25, September 8, September 19, October 1, and October 12, respectively, a period amounting to a little more than 2 weeks longer than when sunrise and sunset alone are considered as delimiting the effective daily light period.

The 18-hour exposures required the use of artificial electric light from sunset, and the duration of this supplemental light was regulated automatically by time clocks and switches, to afford an unbroken period of illumination up to 18 hours each day throughout the season. As the length of day naturally declined after June 21, when the maximum length of day was attained, the period of artificial lighting was increased to compensate for the natural decrements from week to week, just as it was decreased before June 21 as the seasonal day increased.

The artificial supplemental light was supplied by four 200-watt gas-filled tungsten lights fitted with bowl reflectors, one light being placed at each corner of a square metal frame which was 3 feet on the side from center to center of the lights. The lights were so arranged that they could be raised or lowered, and were kept at a distance of 1 foot above the plants. The light intensity of the area beneath this square at a distance of 1 foot amounted to about 300 to 400 foot-candles, as measured by a Weston illumination meter, model 1746, equipped with a Viscor filter designed to measure only visible radiation.

EXPERIMENTAL RESULTS AND DISCUSSION

Great differences were ultimately revealed in the behavior of the various mints tested, some remaining completely vegetative and failing to flower on the shorter lengths of day. There were also marked differences in the production of the prostrate, elongate type of superficial stolons, which serve as very efficient propagating units in the natural extension of the colonies

In table 1, the characteristic behavior of the various species observed is shown.

The two *Monarda* species tested, *M. didyma* (fig. 1) and *M. punctata* (fig. 2), are native American species, the former being known as Oswego tea or American beebalm, some forms of which are highly prized as showy garden ornamentals. These, unlike the *Mentha* species, do not produce long trailing or prostrate stolons with long internodes and reduced leaves.

TABLE 1 — *Time of flowering and growth behavior of species of Monarda and Mentha in response to different constant daily light periods and full day*¹

Species and light period (hours)¹	Date when		Height		Stolons		Growth behavior
	Budded	Flowered	At flowering	Sept 16	Number	Longest	
Monarda didyma			*Inches*	*Inches*		*Inches*	
10	June 24	July 15	24	25	None		Head dry mature Sept 16
12	None						Never flowered, reason unknown
13	June 15	July 5	28	29	None		Heads dry mature Sept 16
14	June 11	July 1	26	25	do		Do
18	June 1	July 2	28	27¹	do		Do
Full day	June 11	July 5	26	28	do		Do
Monarda punctata							
10	June 20	July 23	33	33	None		Do
12	June 15	July 10	26	25¹	do		Do
13	June 11	June 28	22¹	25	do		Do
14	June 15	July 23	28	28	do		Do
18	June 11	July 2	29	30	do		Do
Full day	June 1	July 5	33	33	do		Do
Mentha arvensis							
10	July 20	July 29	24	31	Few	34	Still flowering Sept 16
12	Aug 2	Aug 7	28	34	Very few	34	Still flowering Sept 16 at tips of branches
13	July 20	July 29	28	3	Few	19	Still flowering Sept 16
14	July 22	do	32	40	Many	34	Still flowering Sept 16 stolons of large size vigorous
18	Aug 7	Aug 23	42	46	Few	18	In full flower Sept 16
Full day	do	Aug 27	36	36	Very few	3	Do
Mentha citrata (bergamot mint)							
10	None	None		23	Many	36	Very vigorous leafy plants stolons very abundant long
12	do	do		2	do	21	Vigorous leafy plant
13	Aug 27	Sept 23	28¹	27	Fewer	29	Moderate development of stolons as compared with 10 and 12 hours
14	Aug 23	do	31	30	Rather numerous	10	A considerable number of stolons
18	July 29	Sept 3	31	3	Few	12	Stolons few, short
Full day	Aug 14	Sept 16	31	30	do	30	Just coming into flower Sept 16
Mentha piperita (peppermint)							
10	None	None		19	Many	22	Very leafy plants with numerous stolons
12	do	do		18¹	do		Many stolons as in 10 hour test
13	do	do		21	Fewer	29	Stolons fewer not so long as in 10 and 12 hour tests
14	July 13	July 29	31	34	do	3	These plants barely able to flower stems long vegetative 1 alone producing a few flowers stolons numerous
18	July 12	do	27	28	Very few	16	Very floriferous and in full flower Sept 16 very few stolons
Full day	July 9	do	31	33	Few	17	Last flowering Sept 16 stolons few
Mentha spicata (spearmint)							
10	None	None		2	Many	40	Very leafy vigorous with many long stolons
12	July 1	July 29	25¹	28	do	34	Last flowering Sept 16
13	June 21	July 12	25	29	do	36	Past flowering Sept 16 many long much branched stolons
14	June 19	July 7	28	29	Fewer	40	Last flowering Sept 16
18	June 18	July 8	29	29	Few	9	Past flowering Sept 16 stolons few very short
Full day	June 15	July 10	27	28	do	28	Last flowering Sept 16 few stolons and these of moderate length

TABLE 1.—*Time of flowering and growth behavior of species of Monarda and Mentha in response to different constant daily light periods and full day*—Continued

Species and light period (hours)	Date when—		Height		Stolons		Growth behavior
	Budded	Flowered	At flowering	Sept. 16	Number	Longest	
			Inches	*Inches*		*Inches*	
Mentha piperita var.[1]							
10	July 29	Aug. 5	23	33	Many	38	Still in flower Sept. 16; many vigorous stolons.
12	July 20	July 30	27	40	Fewer	18	Still flowering vigorously Sept. 16.
13	July 13	July 23	30	43	Very few	17	Still flowering vigorously Sept. 16; very few stolons.
14	July 13	July 29	30	40	Few	12	A few flowers at the tips of the flower stems.
18	July 10	July 23	32	42	Very few	11	In full flower Sept. 16; 2 short stolons only.
Full day	July 9	do	28	40	do	7	Still in flower Sept. 16.

[1] Originally from Vick Chemical Co., Greensboro, N. C. Near *Mentha piperita* and perhaps a variety of peppermint (*M. piperita*).

The two species of *Monarda* (table 1) behaved as typical indeterminate or day-neutral plants, since the time of flowering and the increase in height appear to have been little affected by marked changes in length of day. There may have been a tendency to flower somewhat early on the longer light periods, but the tendency was not pronounced.

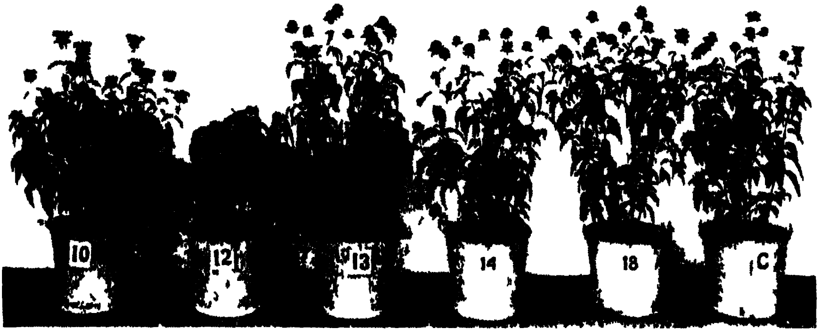

FIGURE 1.—*Monarda didyma* L. exposed to various day lengths. 10-hour day: Budded June 24, flowered July 15 at 24 inches. 12-hour day: Never budded, not normal. 13-hour day: Budded June 15, flowered July 5 at 28 inches. 14-hour day: Budded June 11, flowered July 1 at 26 inches. 18-hour day: Budded June 1, flowered July 2 at 28 inches. Full day (C): Budded June 11, flowered July 5 at 26 inches. Tests began May 2. Photographed July 25.

Several species of *Mentha* (table 1) show decidedly long-day tendencies. This is indicated by the complete failure of flowering as the days were shortened, in the case of *M. citrata*, *M. piperita*, and *M. spicata*.

In *Mentha arvensis* (fig. 3) and in *M. piperita* var. (fig. 4), a possible hybrid, the long-day relationship is scarcely evident, since flowering continued down to the shortest period of 10 hours of constant daily illumination. Even here, however, it is to be noted that flowering, in the case of *M piperita* var , was slightly advanced by the long day of 18 hours In this species a marked retardation of growth took place with respect to the erect branches of the plant on the shorter day

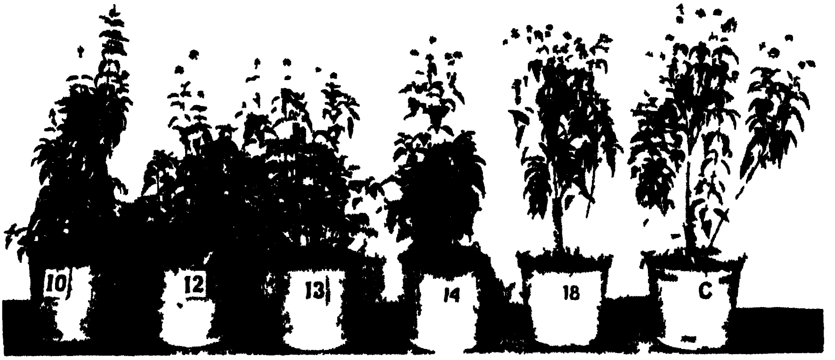

FIGURE 2 *Monarda punctata* L exposed to various day lengths 10 hour day Budded June 29 flowered June 23 at 33 inches 12 hour day Budded June 15 flowered July 10 at 26 inches 13 hour day Budded June 11 flowered June 28 at 22½ inches 14 hour day Budded June 15 flowered July 23 at 28 inches 18 hour day Budded June 14 flowered July 2 at 29 inches Full day (C) Budded June 1 flowered July 5 at 33 inches Tests began May 2 Photographed July 25

lengths but this retardation was accompanied by a decided increase in the formation of stolons both as to number and length This type of stem represents a prostrate form of stem growth tending toward a more purely vegetative expression since these stems are characterized

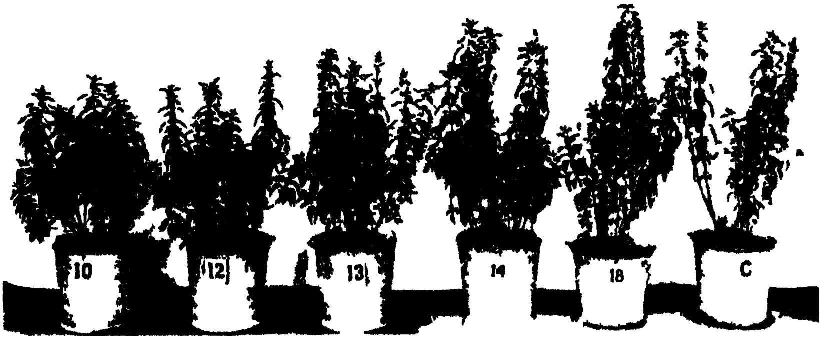

FIGURE 3 *Mentha arvensis* L exposed to various day lengths 10 hour day Budded July 20 flowered July 29 at 24 inches 12 hour day Budded August 2 flowered August 7 at 28 inches 13 hour day Budded July 20 flowered July 29 at 28 inches 14 hour day Budded July 22 flowered July 29 at 32 inches 18 hour day Budded August 7 flowered August 23 at 12 inches Full day (C) Budded August 7 flowered August 27 at 36 inches Tests began May 2 Photographed July 25

by lengthened internodes greatly reduced leaves, and a flowerless condition The last-named tendency would indicate a marked reduction in sexual reproductive energy and to this degree the short days were unfavorable to the development of large vigorous plants and the formation of flowers.

Mentha arvensis gives more indication of being an indeterminate or day-neutral type of plant than any other species of this genus shown in table 1. This strain of *M. arvensis* has given some indication of delay in flowering in response to the longer light periods, and there has been a rather consistent increase in height of stem The number of stolons produced has shown no particular relation to length of day

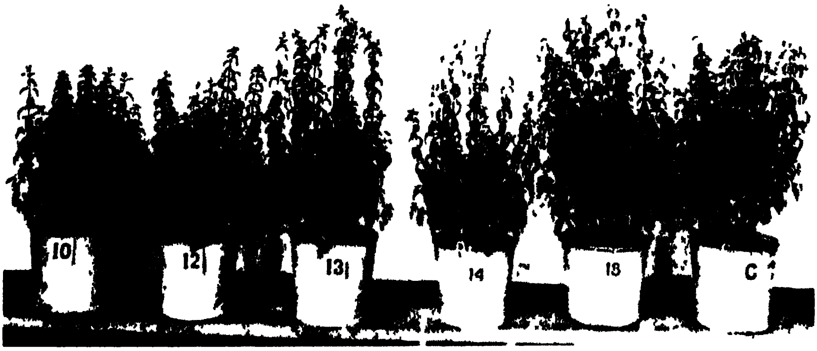

Figure 4 —*Mentha piperita* var exposed to various day lengths Originally obtained from Greensboro, N C., probably a variety of *M. piperita* 10-hour day: Budded July 29, flowered August 5 at 23 inches. 12-hour day: Budded July 20; flowered July 30 at 27 inches 13-hour day: Budded July 13, flowered July 23 at 30 inches 14-hour day: Budded July 13, flowered July 29 at 30 inches 18-hour day: Budded July 10, flowered July 23 at 32 inches Full day (C). Budded July 9, flowered July 23 at 28 inches. Tests began May 2 Photographed July 25.

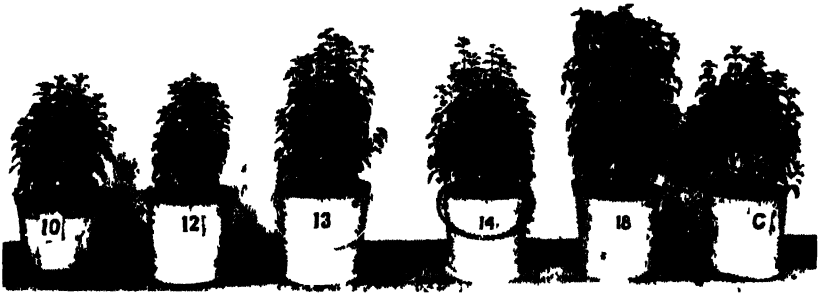

Figure 5 *Mentha citrata* Ehrh exposed to various day lengths. 10-hour day. Never budded 12-hour day: Never budded. 13-hour day: Budded August 27; flowered September 23 at 28½ inches 14-hour day: Budded August 23; flowered September 23 at 31 inches. 18-hour day: Budded July 29; flowered September 3 at 34 inches Full day (C): Budded August 14; flowered September 16 at 31 inches Tests began May 1 Photographed July 25

Mentha citrata (fig. 5) is typically a long-day type of plant, failing to bud or flower on daylight periods of 10 and 12 hours and flowering after great delay in response to 13 hours, 14 hours, and the full length of day. The longest light period of 18 hours hastened flowering in advance of all others, an amount equal to 20 days over those plants experiencing 13 and 14 hours of light daily.

On the shorter lengths of day, more especially those of 10 and 12 hours, where flowering was inhibited, the erect flowerless stems were very leafy, and stolon development was very vigorous, indicating that the energies of growth were almost purely vegetative.

With 18 hours of light daily, vegetative expression was reduced, and sexual reproduction or flowering became the dominant expression, accompanied by weak stolon development, which is always a purely vegetative phase.

Mentha piperita (fig. 6), the true peppermint of commerce, is more pronounced in its long-day tendencies than *M. citrata*, since no buds were produced until a length of day of 14 hours had been experienced. Flowering was entirely inhibited in the tests involving 10, 12, and 13 hours of light daily, no buds being formed in response to these light periods. With 14 hours of light, 18 hours, and full day, buds and flowering occurred at about the same time. Even with 14 hours of light daily, flowers were barely able to develop, one stem alone finally producing a few flowers and all other stems remaining purely vegetative.

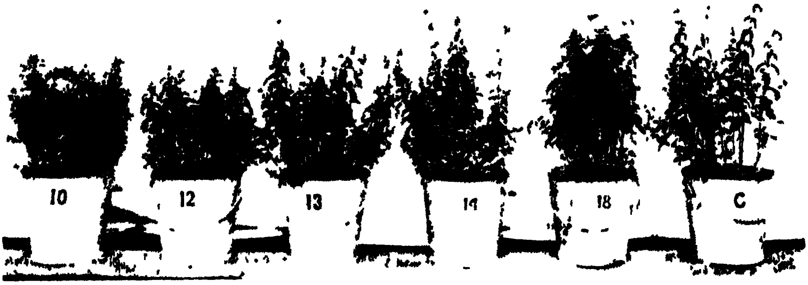

FIGURE 6.—*Mentha piperita* L. (peppermint) exposed to various day lengths. 10-hour day: Never budded. 12-hour day: Never budded. 13-hour day: Never budded. 14-hour day: Budded July 13; flowered July 29 at 31 inches. 18-hour day: Budded July 12; flowered July 29 at 27 inches. Full day (C): Budded July 9; flowered July 29 at 31 inches. Tests began May 2. Photographed August 21.

There was a very noticeable difference in the behavior of the plants experiencing full day as compared with those experiencing 18 hours of light each day. The former had entirely ceased flowering by September 16, whereas the latter were in full flower and very floriferous.

Stolon formation was especially vigorous on the 10- and 12-hour tests, where flower development was inhibited, but had begun to decrease with 13 hours of light. With 18 hours of light and with full day, stolon formation had reached its lowest values, both in number and in length.

Mentha piperita appears to be a species most favored by the long days of high latitudes with respect to flowering and the production of erect leafy stems. In the Washington region flowering was rather brief on the full length of day.

Mentha spicata (*M. viridis* L.) (fig. 7), producing spearmint oil, flowered under all lengths of day except that of 10 hours, when no buds were produced. There appeared to be some delay in flowering with 12 hours of light, a response perhaps to be expected since the short day of 10 hours had yielded a purely vegetative expression. Stolons were very numerous and long on the shorter lengths of day and were much fewer and shorter as the longer daylight periods were experienced. Spearmint gives stronger indications of an adaptation to the shorter days of lower latitudes than peppermint (*M. piperita*) has shown.

The plant designated *Mentha piperita* var. flowered under all lengths of day in the tests but with perhaps a slight delay with 10 hours of light daily. On this short light period there is evidence that vegetative expression was being favored, since stolon development was heaviest and the longest runners were produced. With each increase in length of day above 10 hours, stolon development declined until with 18 hours of light only two short runners developed.

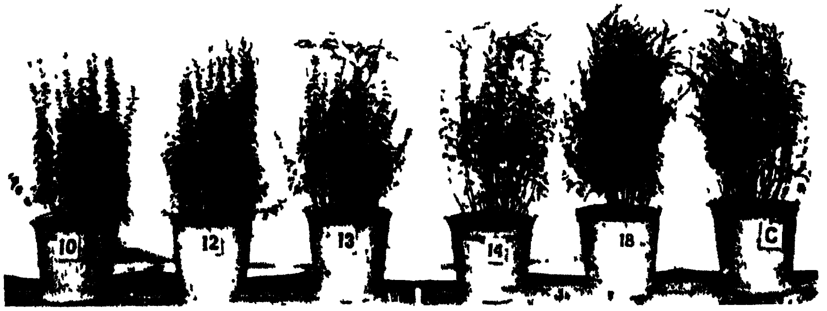

FIGURE 7. *Mentha spicata* L. (*M viridis* L.) (spearmint) exposed to various day lengths 10-hour day. Never budded. 12-hour day: Budded July 1, flowered July 29 at $25\frac{1}{2}$ inches. 13-hour day: Budded June 21, flowered July 12 at 25 inches. 14-hour day: Budded June 19, flowered July 7 at 26 inches 18-hour day: Budded June 18, flowered July 8 at 29 inches. Full day (C): Budded June 15; flowered July 10 at 27 inches Tests began May 1. Photographed August 21.

If this mint represents a variety of *Mentha piperita*, it would appear that the wide range of flowering, extending far down into the shortest light periods, is more nearly like the behavior of *M. arvensis* than that of *M. piperita* (table 1) and indicates the marked differences that closely related varieties of a species may show in their reaction to the factor of length of day.

CONCLUSIONS

The present paper shows the day-length requirements of a number of strains of species of *Monarda* and *Mentha* grown at the Arlington Experiment Farm, Arlington, Va. Some of these are of Old World origin, and a number, including *Mentha spicata* and *M. piperita*, are thoroughly naturalized in the East.

Monarda punctata, *Mentha spicata*, and *M. piperita* occur wild in the District of Columbia; *Monarda didyma* occurs wild in the adjacent area of Virginia.

Some of these mints have very definite length-of-day requirements for flowering, and the various species differ widely in this respect. Some are obviously best adapted to northern regions with long days, while others can flower readily in lower latitudes where much shorter summer days prevail.

The species *Monarda punctata* and *M. didyma* have flowered readily under all light periods and behave as indeterminate or day-neutral plants, their flowering being little affected by the length of the daily light periods. On the basis of their readiness to flower in response to various day lengths, ranging from 10 to 18 hours, it should be expected that both species would have a wide natural range of distribution southward and northward. That such is the case is shown by the fact that *M. didyma* is found from Quebec, Canada, to Florida, and *M. punctata* from New York to Florida and Texas. It is probable that at the limits of their range, in both the extreme north and the extreme south, the temperature factors may play an important part in their delimitation.

The several species of *Mentha*, for the most part, are typical long-day plants in their day-length responses, since some flowered more quickly when the longer lengths of day were experienced and others flowered only under these longer day lengths. Of the five strains or species tested, *M. piperita* showed the most pronounced requirement for long days, since flowering was not initiated even with 13 hours of light daily and flowers were barely able to form when the plants were given 14 hours of light daily. In the case of *M. citrata*, the lower limits of flowering were shown with 12 hours of light daily, and plants of *M. spicata* failed to flower with 10 hours of light daily.

It is important to recognize the fact that the plants of the various species tested probably represent particular strains in all instances and that these results would not necessarily apply to all other strains of these species found in this country or in the Old World.

As has been indicated, most of the strains of the *Mentha* species dealt with in this paper have shown more or less pronounced long-day requirements so far as free flowering is concerned. The recognition of this fact is of much importance to the plant breeder who may hope to hasten the flowering of certain species or to synchronize their flowering to best advantage for purposes of ready crossing by modifying the daily light period. It is obvious that shortening the days to hasten flowering in some of these species, as one plant breeder interested in mint breeding informed the writer he had done, is anything but the correct procedure.

The writer's studies of these mints have been concerned only with the ecology of growth and flowering, but the percentage yields of aromatic oil and its composition in relation to the various lengths of day would also have been of considerable interest.

It is evident that the various species have shown strongly contrasting responses to the various lengths of day, and it is true generally that where flowering was inhibited on the shorter days stolon development and elongation were favored.

The species of *Mentha* for the most part produce two types of stems, one of erect habit bearing the normal leafage and capable of producing flowers under suitable conditions; the other a prostrate, stoloniferous, nonflowering type, with long internodes and small leaves, which may grow to excessive length and root readily along its course when in contact with the ground. It is obvious that the latter is a strictly vegetative, propagative type of stem, which serves to extend the colonies into new areas.

Such evidence as has been secured would indicate that, with suppression of the flowering tendency under shortened day lengths, the stolon type of stem development is favored. An undue accentuation of this type of stem, with restriction of erect stem growth, obviously would tend to lower the production of plant material per acre and consequently the yield of aromatic oil. It would appear, then, that maximum yields, percentage composition being equal, would obtain in those areas most favorable to maximum erect stem growth, which to a greater or less degree is related to favorable length of day. For optimum conditions of flowering and for early flowering long days are essential. This is probably one of the principal reasons why peppermint (*Mentha piperita*) succeeds best in northern latitudes.

SUMMARY

The length-of-day requirements of a number of mints of the genera *Monarda* and *Mentha* of the family Labiatae were studied, the range of photoperiods being 10, 12, 12½, 13, 13½, 14, 14½, 18 hours, and full day, at Arlington, Va., at approximately lat. 39° N.

Natural daylight was used for all photoperiods except that of 18 hours, this being much in excess of the longest natural day at Washington, D. C., which on June 21 is 14.9 hours.

For photoperiods of 10 to 14½ hours, inclusive, the plants were kept in ventilated lightproof houses for definite periods each day to obtain the desired number of hours of daylight exposure.

For the 18-hour photoperiod, artificial supplemental light was afforded the plants by four 200-watt gas-filled tungsten lights fitted with bowl reflectors, one light being placed at each corner of a square metal frame 3 feet on a side from center to center of the lights. These lights were kept at a distance of 1 foot from the plants by raising the supporting metal frame as needed. The light intensity thus afforded was about 300 to 400 foot-candles as measured by a Weston illumination meter, model 1746, equipped with a Viscor filter measuring visible radiation only.

The species studied were *Monarda didyma*, *M. punctata*, *Mentha arvensis*, *M. citrata*, *M. piperita*, *M. spicata*, and a variety of *M. piperita*.

Most of these mints require long days for early profuse flowering, but the various *Mentha* species, which are characterized by the formation of long stolons under some conditions, show accentuation of the stoloniferous habit in some instances on the shorter days.

The two *Monarda* species, *M. didyma* and *M. punctata*, are day-neutral or indeterminate in their behavior, since the most extreme differences in the length of the photoperiod had very little effect on the height and time of flowering.

Mentha citrata, *M. piperita*, and *M. spicata* have shown the most decided long-day tendencies, since flowering was inhibited entirely on shortened days. Of these, *M. piperita* was most favored by long days, since only the photoperiods of 14 hours, 18 hours, and the full day allowed buds and flowers to appear.

Mentha arvensis and a variety of *M. piperita* obtained from Greensboro, N. C., produced flowers even in response to the shortest photoperiod of 10 hours, and to this extent both are inclined to be indeterminate or day-neutral in their length-of-day responses.

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PROGRESS OF GERMINATION OF SEED OF DIGITARIA AS INFLUENCED BY GERMINATION TEMPERATURE AND OTHER FACTORS[1]

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INTRODUCTION

That different kinds of seeds respond differently to germination temperature has long been recognized. The work of Crocker (*1*, *2*)[2] indicated that these responses are not only associated with the behavior of the cells of the embryo, but are complicated by the coat restrictions. It may be assumed that some fundamental principles are involved in these varied responses of different seeds. A knowledge of the detailed behavior of many kinds of seeds that have different adaptations is needed before valid generalizations can be made. A study of the well-recognized special germination requirements of the seeds of many weeds should furnish information of value in solving the general problem of seed germination. Also, a knowledge of the factors influencing the germination of seeds of specific weeds should aid in their control. The seeds of the two common crabgrasses *Digitaria ischaemum* (Schreb.) Muhl. and *D. sanguinalis* (L.) Scop. were selected for study.[3]

Only incidental references in the literature to the germination requirements of *Digitaria* have been found. Weber and Kirchner (*15*, *p. 282*) mention that germination of the seed of *D. sanguinalis* is slow and that light is required. Heating the seed at 35° C. had a beneficial effect in hastening germination. Edwards (*4*) has reviewed the literature on the relations of temperature to germination of various seeds, dealing mostly with the response to temperatures maintained at a constant level; Harrington (*8*) has referred to the early work on the effect of daily alternation of temperatures on seed germination; and Morinaga (*13*) and many others have presented work on this phase of temperature relations. The beneficial effect of prechilling, i. e., holding the moist seeds at a temperature slightly above freezing before subjecting them to ordinary temperatures for germination, was first described by Davis and Rose (*3*). The method has been used by many workers for various types of retarded germination of seeds. The changed response of many seeds with age after harvest has been noted by various writers. Harrington (*7*) has reviewed the early literature on the afterripening of cereals. Kearns and Toole (*10*) have recently followed the change with age of the response of fescue seed to alternations of temperature.

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[1] Received for publication December 27, 1940 This investigation was conducted in the former Division of Seed Investigations, Bureau of Plant Industry

[2] Italic numbers in parentheses refer to Literature Cited, p 90.

[3] A preliminary report of some portions of this study was given before the Botanical Society of America in December 1938

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MATERIAL AND METHODS

Seed collections were made in 1935, 1936, 1937, and 1938 from growing *Digitaria* plants by lightly rubbing the mature inflorescences to remove only the mature seeds. The seed was cleaned to remove chaff and undeveloped fruits and stored in paper bags in the laboratory.

The "seed" as used in these germination studies consisted of the mature fruit or caryopsis enclosed by the close-fitting cartilaginous lemma and palea and the persistent second glume and sterile lemma. Because of the uneven ripening of the seed of these grasses and the tendency of the seed to fall from the plant as soon as it was fully mature, there undoubtedly was a considerable and variable degree of immaturity in the material used.

All germination tests were carried out on moistened paper toweling in Petri dishes. Alternation of temperature was obtained by transferring the dishes from one controlled-temperature chamber to the other. The first-mentioned temperature of an alternation was maintained for approximately 18 hours and the second for 6 hours each day. Seedlings that had developed a definite root and plumule were counted and removed every 7 days, and results usually are reported for 28-day periods.

The temperature of germination chambers above room temperature was maintained by thermostatically controlled electric lamps within the chambers. No attempt was made to exclude light except in special tests in which the Petri dishes were enclosed in tin boxes. Definite exposure to light was made only at the condition designated "20° to 30° with light," in which, for the approximately 6-hour period at 30° C., the germination tests were carried on in a glass-enclosed chamber in a north window.

EXPERIMENTAL RESULTS

Preliminary tests in 1935 at various temperatures indicated that freshly harvested seed of both *Digitaria ischaemum* and *D. sanguinalis* would remain for several weeks with practically no germination under conditions ordinarily favorable for germination, but that when germination finally started it progressed steadily although slowly until practically all (95 98 percent) the viable seed had germinated, which required several months. The final germination was about the same when the seed was maintained at different daily alternations of temperature, but the course of germination was very different. Prechilling the seed shortened the time before germination started and hastened the rate of germination. Tests started after the seed had been stored dry for 2 months in the laboratory showed a marked change in the course of germination.

These preliminary results led to a more detailed study (1) of the comparative response of the two species to a series of daily alternations of temperature, (2) of the change of this response as the seed aged, and (3) of the response of the seed after prechilling for various periods.

EFFECT OF SEVERAL TEMPERATURE ALTERNATIONS ON GERMINATION OF SEED OF DIGITARIA ISCHAEMUM

Freshly Harvested Seed

Germination tests were started within 2 days after harvesting in the fall of 1936, 1937, and 1938, and four alternations of temperature

(20° to 40° C., 20° to 35°, 20° to 30°, and 15° to 25°) were used. The results of these tests, as presented in table 1, show considerable variation in the germination of the different samples at a given temperature. The relative progress of each sample is very similar for the several temperatures, however, and it is believed that the means for the four samples (fig. 1) represent with reasonable accuracy the relative response to these temperature conditions of the freshly harvested seed of *Digitaria ischaemum*.

The general forms of the curves for germination at alternations 20° to 40° C., 20° to 35°, and 20° to 30° are similar. A more or less lengthy initial period without germination or with only slight germination was followed by a period of comparatively rapid germination, after which the rate became much slower and the germination of the remaining seeds continued over a very long period. Even during the period of comparatively rapid germination, the mean rate (1 to 1.5 percent per day) was very slow in comparison with the rate of germination of most crop seeds.

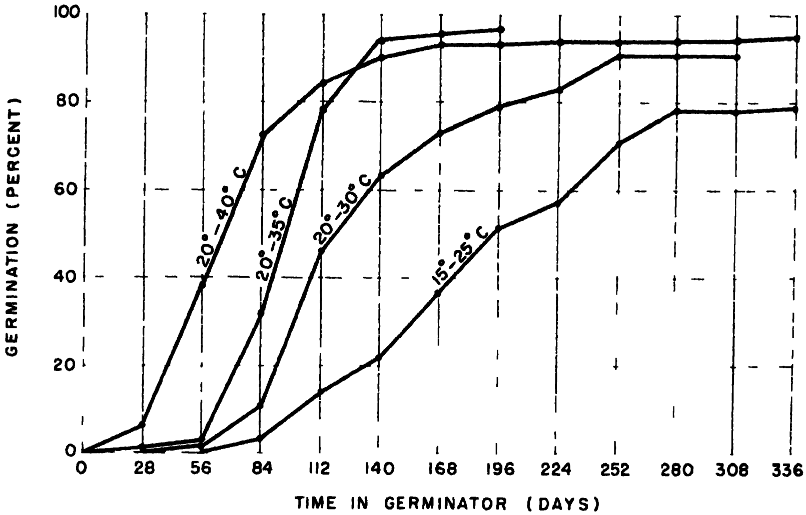

FIGURE 1. Mean germination of four samples of freshly harvested seed of *Digitaria ischaemum* at four temperature alternations.

Rapid germination started first at the 20° to 40° C. alternation, about 4 weeks later at 20° to 35°, and after another 3 or 4 weeks at 20° to 30°. When well started, germination was for a time at about the same rate for these three conditions. Although germination started much later at the 20° to 35° than at the 20° to 40° alternation, a smaller percentage of seed remained ungerminated at the former temperature alternation when the rate of germination slowed down in the later part of the test. At the conclusion of most of the individual tests at 20° to 40° a few apparently sound seeds remained, and this in general was not true at 20° to 35°. At 20° to 30° the rate of germination fell markedly after about 50 percent of the seeds had germinated. The difference in germination among samples was greater at 20° to 30° than at 20° to 40° or at 20° to 35°.

TABLE 1 *Summary of course of germination of 4 samples of Digitaria ischaemum at 4 temperature alternations when tested immediately after harvest*

Temperature alternation (° C)	Sample No	Year of harvest	Seeds tested	Cumulative percentage germination after indicated number of days											
				28	56	84	112	140	168	196	224	252	280	308	336
			No	Pct	Pct	Pct	Pct	Pct	Pct	Pct	Pct	Pct	Pct	Pct	Pct
20 to 40	761848	1936	200	0	[illegible]4	87	9[illegible]	94	9[illegible]	9[illegible]					
	766396	1937	200	11	36	76	89	93	94	95	96				
	766401	1937	200	1[illegible]	11	66	79	87	94	95					
	769356	1938	400	0	21	64	71	87	88	89	90	91	91	92	93
Total or mean			1 000	6	38	73	84	90	93	93	94	94	94	94	9[illegible]
20 to 35	761848	1936	400	0	0	30	78	93	94	9[illegible]					
	766396	1937	200	2		3[illegible]	87	9[illegible]	96	97					
	766401	1937	200	1		28	73	[illegible]	98	9[illegible]					
	769356	1938	100	0	1	38	74	92	97	97					
Total or mean			1 200	1	3	32	78	94	9[illegible]	97					
20 to 30	761848	1936	200	0	0	[illegible]	30	[illegible]1		[illegible]9	60	87	88	89	
	766396	1937	200	0	3	20	[illegible]1	[illegible]9	[illegible]3	79	81				
	766401	1937	200	1	2	13	[illegible]1	72	77	8[illegible]	89	9[illegible]			
	769356	1938	100	0	0	[illegible]	[illegible]4	73	86	90	98				
Total or mean			1 000	0	1	11	4[illegible]	[illegible]3	73	7[illegible]	83	91	91	91	
15 to 25	761848	1936	200	0	0	1	8	12	30	[illegible]7	[illegible]8	[illegible]1	91	92	
	766396	1937	200	0	0	[illegible]	[illegible]8	13	31	[illegible]0	[illegible]6	[illegible]0	[illegible]		
	766401	1937	200	0	0	1	7	17	3[illegible]	63	73	80	93		
	769356	1938	400	0	0	[illegible]	33	[illegible]4	[illegible]1	[illegible]8	[illegible]3	[illegible]	[illegible]	[illegible]	[illegible]
Total or mean			1 000	0	0	3	14	22	[illegible]3	[illegible]2	[illegible]	71	78	78	[illegible]

At the 15° to 25° C alternation, germination was not only slow to start but it progressed at a much slower rate than under the other temperature conditions and the final germination was less. The means of the four samples indicate a fairly uniform rate of germination, approximately 2 75 percent per week from the twelfth to the fortieth week The total germination obtained at the 15° to 25° alternation was very different for the four samples This may in part have been due to undetected differences in germination conditions in the different years On the other hand, samples 766396 and 766401 were in the same chambers at the same time and yet germinated very differently at this temperature alternation, although these same samples behaved essentially alike at 20° to 40° and at 20° to 35°.

STORED SEED

Germination tests started September 23, 1935 (day of seed collection), November 22, 1935, January 13, 1936, and September 30, 1936, on sample 758522, gave results (fig 2) for the temperature alternation 20° to 35° C that indicated a progressively earlier start of germination for the successively later tests When the seed was a year old, germination was completed in 7 days, whereas with freshly harvested seed germination extended over a period of 224 days

With seed collected in 1936, tests in September and again in December indicated a similar change in the response of the seed In 1938 it was planned to follow closely the changing germination response of the seed when stored dry in the laboratory Tests were started (sample 769356) the day the seed was collected, September 8, and at weekly intervals thereafter at 20° to 40° C., 20° to 35°, 20° to 30°, and 15° to 25°. By October it was realized that the interval between tests was too short to indicate definite change, but the supply of seed was so nearly exhausted that the series could not be extended. The remaining seed was tested on February 2, 1939. The results of these tests (table 2) suggest that at 20° to 30° and at 20° to 35° the seed germinated better when tested the day it was collected than when tested 1 and 2 weeks later; however, the differences are not large enough or consistent enough to be more than suggestive. With this possible exception the results from September 8 through October 6 for 15° to 25° and 20° to 30°, and through October 13 for 20° to 35° and 20° to 40°, are fairly uniform and show at least no marked change in the response of the

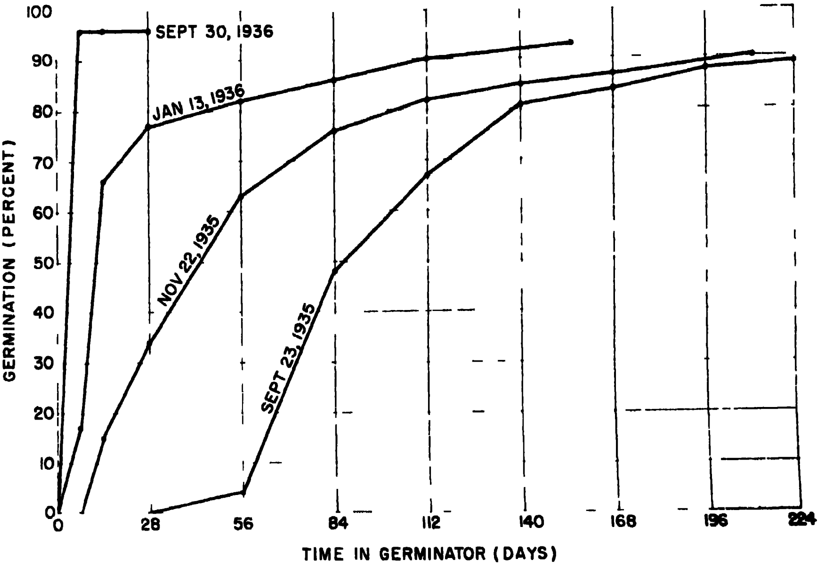

FIGURE 2.—Progress of germination of seed of *Digitaria ischaemum* at 20° to 35° C. alternation when tested at time of harvesting and at different dates thereafter. Sample 758522, collected September 23, 1935.

samples over this period. In February 1939 germination was greater in 14 days than in 112 days for the earlier tests and there was very little difference in the course of germination at the three temperature alternations 20° to 40°, 20° to 35°, or 20° to 30°.

With seed 1 to 3 years old (sample 758522) germination was essentially the same at the four temperature alternations except for slightly slower germination at 15° to 25° C. With seed 1 year old germination at 20° to 40°, 20° to 35°, and 20° to 30° was approximately 95 percent in 7 days and was completed (96 to 99 percent) in 14 days. At 15° to 25° germination was approximately 95 percent in 14 days and was completed in 14 to 21 days. Plumules were noticeably shorter in 5 days at 20° to 40° than at 20° to 35°. There was no evidence of further change in response of the seed after 1 year.

TABLE 2.—*Summary of germination at different temperature alternations of seed of Digitaria ischaemum when tested at successive periods after harvest*

[Sample 769356, collected Sept. 8, 1938. 400 seeds used for each test]

Temperature (° C.)	Period of germination count	Germination [1] in tests started Sept. 8, 1938	Sept. 15, 1938	Sept. 22, 1938	Sept. 29, 1938	Oct. 6, 1938	Oct. 13, 1938	Mean	Feb. 2, 1939
	Days	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
20 to 40	14	0	0	0	0	1	0	0	77
	28	0	1	0	2	2	1	1	81
	56	21	13	17	22	25	14	19	87
	84	64	58	48	48	55	56	55	87
	112	74	74	68	63	74	76	72	89
	140	87	89	87	80	90	93	88	90
	168	88	93	91	88	91	93	91	96
	196	89	94	94	93	93	95	93	97 (3)
	224	90	97	95	93	93	95	94	
	252	91	97	95	93	93	95	94	
	280	91	97	95	94	93	95	94	
	308	92	97	95	94	94 (2)	96	95	
	336	93 (2)	97 (1)	97	95 (1)			95	
20 to 35	14	0	0	0	0	0	1	0	73
	28	0	0	0	0	0	1	0	83
	56	1	0	0	0	1	2	1	86
	84	38	29	22	27	31	11	31	88
	112	71	69	61	67	66	77	69	89
	140	92	90	81	91	91	92	90	89
	168	97	97	93	93	95	97	95	92
	196	97		95	98	98	98	97	94 (3)
20 to 30	14	0	0	0	0	0		0	65
	28	0	0	0	0	0		0	81
	56	0	0	0	0	0		0	84
	84	6	1	1	4	8		4	87
	112	51	38	30	15	4		11	89
	140	73	51	54	62	64		61	92
	168	86	63	63	69	68		70	93
	196	90	67	72	77	77		77	92 (3)
	224	98	77	81	83	91		88	
	252		84	81	94	92		91	
	280		84	85	94	92		91	
	308		84	85	94	95)		91	
	336		84 (13)	85 (13)	94 (1)			91	
15 to 25	28	0	0	0	0	0		0	
	56	0	0	1	2	3		1	
	84	7	6	11	10	16		10	
	112	13	34	36	37	28		4	
	140	45	46	45	51	43		46	
	168	51	53	51	56	48		52	
	196	58	56	51	60	52		56	
	224	63	62	59	66	[illegible]		61	
	252	63	69	65	69	60		65	
	280	64	71	66	70	60		66	
	308	64	71	66	73	62		67	
	336	65	71	67	75			68	

[1] Figures in parentheses after final percentages show approximate percentage of apparently sound seed remaining when test was discontinued.

PRECHILLED SEED

In studying the effect of prechilling, the seeds were placed on moistened paper toweling in Petri dishes, and these were held in a cold chamber at approximately 3° C. (2° to 5°) for various periods before they were removed to the usual germination chambers. The germination count was reckoned from the time the seed was moistened and placed at the low temperature. No germination occurred while the seeds were at the low temperature.

A few representative tests are presented from among the many carried out. The course of germination of freshly harvested seed at 20° to 35° C. after prechilling is shown in figure 3 for two samples collected in different years.

The freshly harvested seed of the two samples behaved very much alike. Prechilling for 2 weeks caused a small proportion of the seeds to germinate more quickly, but after these had germinated the further progress of germination of the prechilled and nonchilled tests was similar, and there was a definite suggestion of a lower final germination of the seed that had been prechilled. Treatment of the freshly harvested seed at a low temperature for 4 weeks caused a much larger proportion of the seeds to germinate rather promptly after they were subjected to a 20° to 35° C. alternation of temperature, but even after this period of treatment a certain proportion of the seeds was at least as slow to germinate as the untreated seeds. After prechilling for 8 weeks, over 90 percent of the seeds germinated within a week after removal to the higher temperature condition, and germination of the viable seeds was completed much earlier than that of the untreated seed.

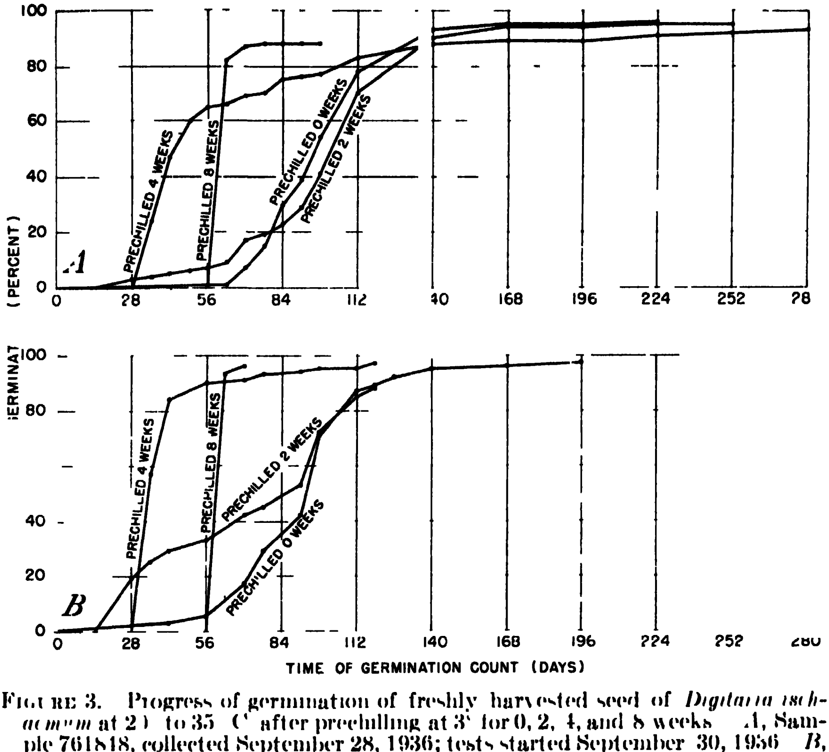

FIGURE 3. Progress of germination of freshly harvested seed of *Digitaria ischaemum* at 20° to 35° C. after prechilling at 3° for 0, 2, 4, and 8 weeks. *A*, Sample 761818, collected September 28, 1936; tests started September 30, 1936. *B*, Sample 766396, collected September 28, 1937; tests started September 29, 1937.

When the behavior of fresh seed is compared with that of seed from the same sample after approximately 2 months' dry storage (figs. 3, *A*; 4), it is noted that the older seed after 2 weeks' prechilling germinated almost as promptly as the fresh seed after 8 weeks' treatment, although, even with this older seed, a longer treatment (4 weeks) at a low temperature brought about prompt germination of a slightly greater proportion of the seeds.

The seed collected September 8, 1938 (sample 769356), showed, as previously noted, no definitely significant difference in behavior when tested without prechilling at weekly intervals from September 8 to October 13. However the results of tests made at these same intervals with seed that had been prechilled for 2 weeks after dry storage for 0 to 5 weeks (table 3) showed a progressively greater germination at the earlier germination counts as the seed became older. The germination in 28 days of seed that had been stored dry for 4 weeks was 44 percentage units higher than that of the seed tested after 3 weeks in dry storage. Seed that had been stored 5 weeks and then prechilled for 2 weeks germinated almost as promptly as seed that had been stored dry for 21 weeks but not prechilled. These differences had largely disappeared by the one hundred and fortieth day of germination count.

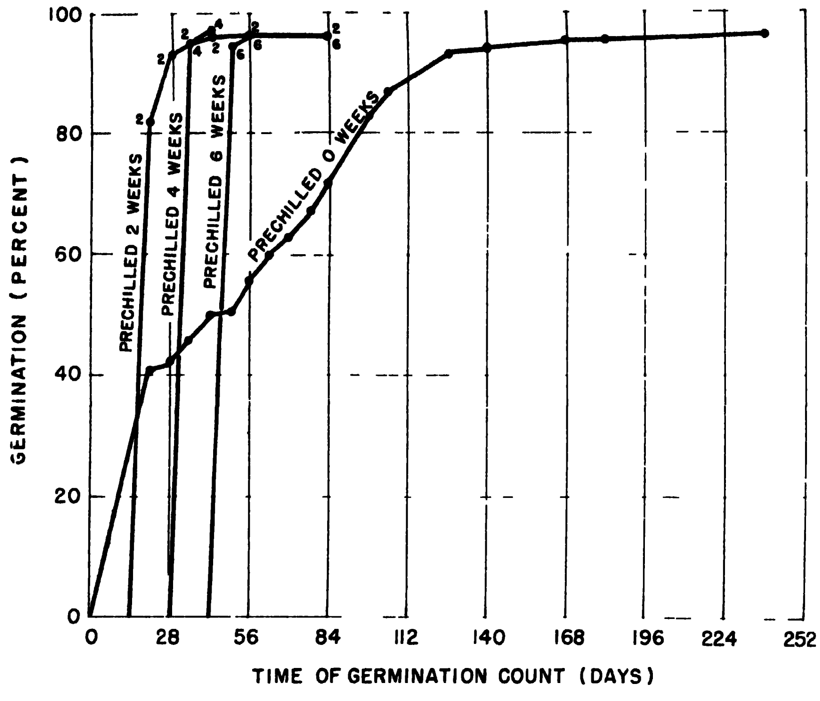

Figure 4. Progress of germination of older seed of *Digitaria ischaemum* at 20° to 35° C. after prechilling at 3° for 0, 2, 4, and 6 weeks Sample 761848, collected September 28, 1936; tests started December 9, 1936.

The results stated above on the effect of prechilling have been based on subsequent germination at 20° to 35° C. A comparison of the progress of germination at four temperature alternations after prechilling the seed 2, 4, and 8 weeks (fig. 5) shows that the response of prechilled seed to temperature of germination was similar to that shown by untreated freshly harvested seed. However, the differences in germination response at the four alternations were less with the longer periods of prechilling.

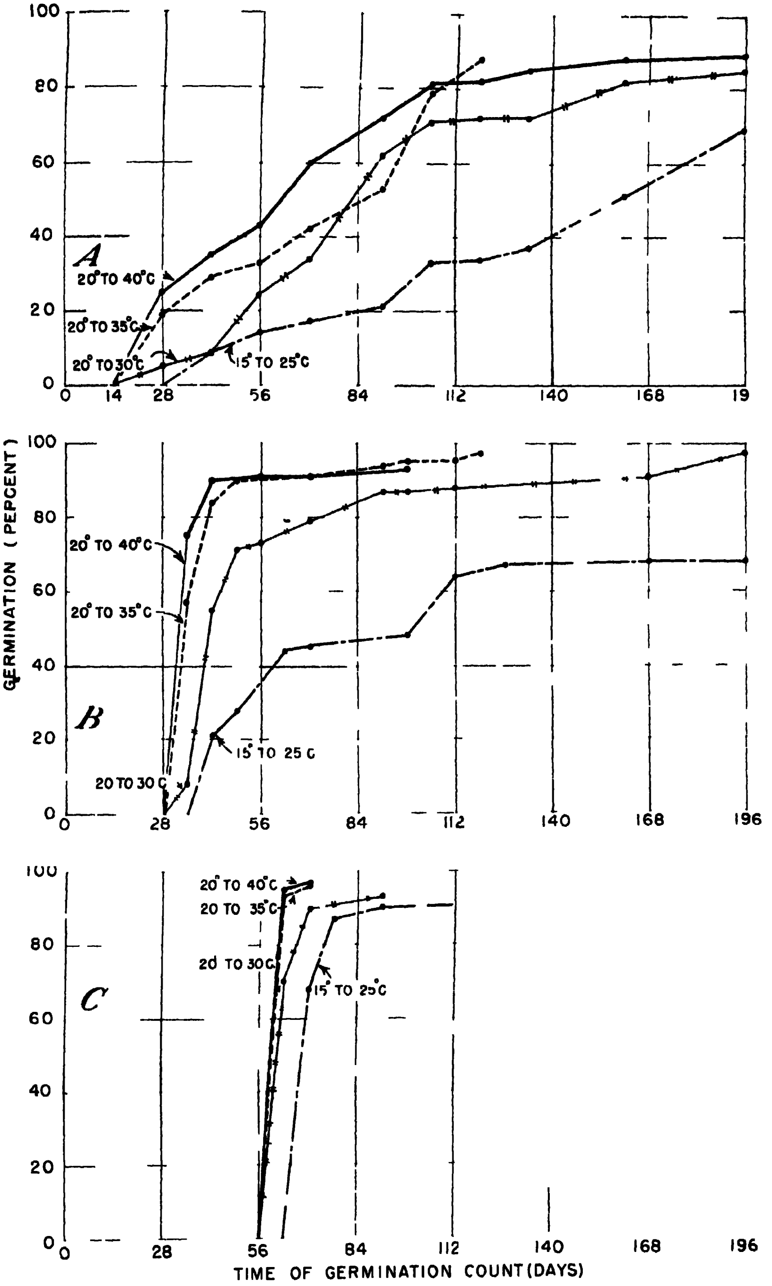

FIGURE 5.—Progress of germination of seed of *Digitaria ischaemum* at four temperature alternations after prechilling at 3° C. for (*A*) 2 weeks, (*B*) 1 week, and (*C*) 8 weeks. Sample 766396, collected September 28, 1937, tests started September 29, 1937.

TABLE 3.—*Effect of prechilling at 3° C. for 2 weeks on the subsequent germination at 20° to 35° of seed of Digitaria ischaemum stored dry for 0, 1, 2, 3, 4, or 5 weeks*

[Sample 769356 collected Sept. 8, 1938; 400 seeds used for each test]

Period of germination count (days)	Germination after prechilling for 2 weeks at 3° C. after dry storage for—						Germination without prechilling after dry storage for	
	0 week	1 week	2 weeks	3 weeks	4 weeks	weeks	0 weeks	21 weeks
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
28	8	8	17	31		71	0	83
56	16	13	27	71	80	80	1	86
84	36	29	2	68	81	81	31	88
112	64	66	67	[illegible]	82	83	69	89
140	87	81	8	93	88	91	90	89
168	93	97	[illegible]	96	91	97	9	92
196	9	98	98	99	99	97	97	94

Mean of the 6 tests started at weekly intervals from Sept. 8 to Oct. 13, inclusive.

MISCELLANEOUS OBSERVATIONS

In the course of the above-described work other observations were made that seem worth recording.

In the general routine of testing, additional tests were made in which the paper toweling was moistened with a 0.2-percent solution of potassium nitrate instead of water. The most striking results were obtained with freshly harvested seed of sample 761848 (fig. 6). There was a distinct hastening of germination by the use of potassium nitrate at the 15° to 25° C. and 20° to 30° temperature alternations and an equally strong retardation at the 20° to 35° and 20° to 40° alternations.

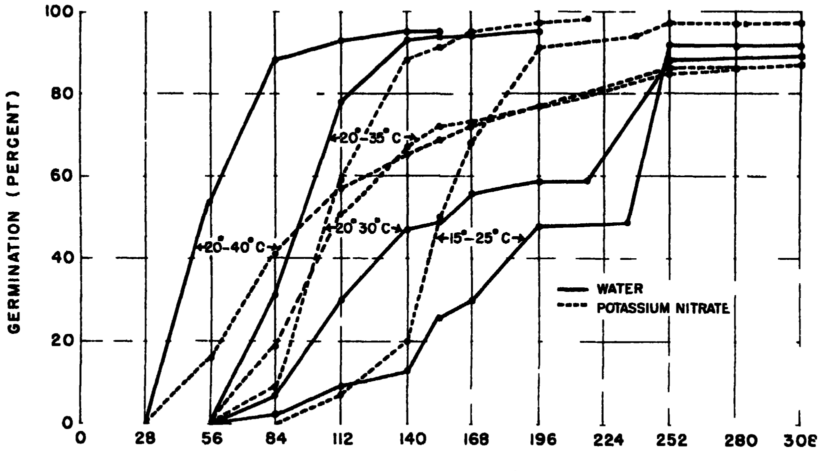

FIGURE 6.—Progress of germination of freshly harvested seed of *Digitaria ischaemum* at four temperature alternations, on paper toweling moistened with water and with a 0.2-percent solution of potassium nitrate at each alternation. Means of 200 seeds for each test, except 400 seeds used at 20° to 35° C. Sample 761848.

Difference in germination between seeds treated with water and seeds treated with potassium nitrate was not apparent at some of the temperature alternations with some samples, but whenever a difference occurred it was in the direction indicated in figure 6.

A detailed study of the effect of light on germination was not made. Most of the tests received some light from electric lamps used to heat the germination chambers and also from brief exposure during transfer from one temperature to another and during the counting of the germinated seedlings. Some of the series received definite exposure to diffuse daylight during a portion of each day. In a few instances there were parallel tests in which direct light exposure was compared with total light exclusion by enclosing the tests in a tin box. The results were somewhat erratic and inconclusive, and no definite or striking effect of light on the germination of seed of *Digitaria ischaemum* was observed.

It is usually assumed that retarded germination such as reported here for *Digitaria* is caused by restrictions of the seed or fruit coats; therefore, some tests were conducted to determine the effect of mechanical scarification and treatment with concentrated sulfuric acid. Rubbing the fruits with emery paper caused a slight hastening of germination in the first few weeks, but about two-thirds of the seeds were badly injured by the treatment. After treatments for ½ minute and for 1 minute with concentrated sulfuric acid, approximately 60 percent of the seed germinated in 28 days, at which time germination had hardly started in the control. After 19 weeks in the germinator there was very little difference between the germination of the treated and the untreated seeds. Treatment for 2 minutes caused marked injury.

EFFECT OF SEVERAL TEMPERATURE ALTERNATIONS ON GERMINATION OF SEED OF DIGITARIA SANGUINALIS

Freshly Harvested Seed

A summary of the germination of several samples of seed of *Digitaria sanguinalis* collected in different years and tested within a few days after harvest at several temperature alternations is given in table 4. Germination of the several samples at any one of the temperature alternations was very variable. This variation is evident in figure 7, which shows the progress of germination at four temperature alternations of two samples collected the same year and tested at the same time. (See also fig. 11.) In spite of this variation among samples, the relative response of each of the samples to the different temperature conditions was much the same (table 4). The progress of germination at different temperature alternations, based on the means of all available information for each alternation, is shown in figure 8.

In general, germination was most rapid and reached the highest final value at 20° to 30° C. with light. Germination at 20° to 35° progressed at a somewhat slower rate, and final values were slightly lower. At 20° to 40° the start of germination was delayed and its progress was at a definitely slower rate than at the two conditions mentioned above, and for most of the samples germination of the viable seed was not completed in the time the tests were conducted. Germination of three samples tested at 15° to 25° was extremely slow. The behavior of the freshly harvested seed at the other alternations used will be discussed later, in connection with the effect of exposure to light (p. 83).

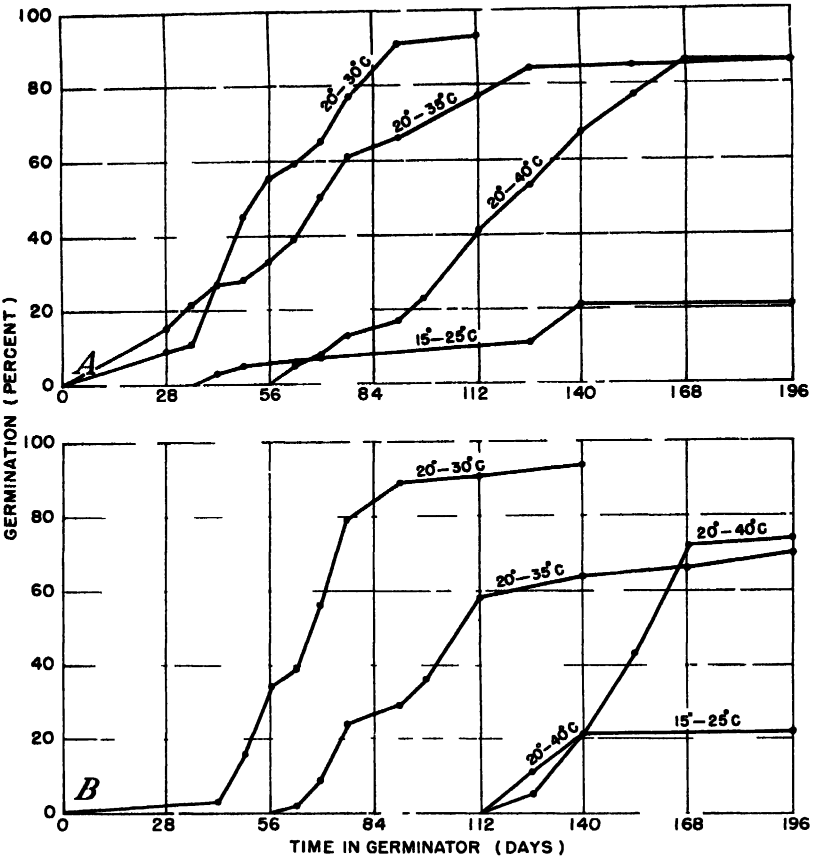

FIGURE 7.—Progress of germination of freshly harvested seed of *Digitaria sanguinalis* at four temperature alternations, showing variable response of (*A*) sample 766403 and (*B*) sample 766404. Seed collected September 29, 1937; germination started October 2, 1937.

TABLE 4.—*Course of germination of various samples of seed of* Digitaria sanguinalis *at different temperature alternations when tested immediately after harvest*

Temperature alternation (° C.) and light condition	Sample No.	Year of harvest	Seeds tested	Cumulative percentage germination after indicated number of days: 28	56	84	112	140	168	196	224	252	280	308	336
			Number	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
20 to 40	761849	1936	200	0	16	43	60	80	83	83	85	86	86	92	
	766403	1937	100	0	3	15	41	67	86	86	86				
	766404	1937	200	0	0	0	2	21	72	74	75				
	769357	1938	400	0	2	11	33	54	61	65	73	74	74	74	75
Total or mean			900	0	5	17	34	55	75	77	80	80	80	82	
20 to 35	778523	1935	200	0	33	62	87	96	97						
	761849	1936	400	0	29	72	84	89	91	91	91	94			
	766403	1937	100	1	33	63	77	84	85	86	86				
	766404	1937	200	0	0	26	58	64	66	70	70				
	769357	1938	400	0	14	55	76	84	86	86	88	91	91	93	93
Total or mean			1,300	0	22	56	76	83	85	86	87	88	88	88	

TABLE 4.—*Course of germination of various samples of seed of Digitaria sanguinalis at different temperature alternations when tested immediately after harvest*—Cont.

Temperature alternation (°C) and light condition	Sample No.	Year of harvest	Seeds tested	Cumulative percentage germination after indicated number of days											
				28	56	84	112	140	168	196	224	252	280	308	336
			Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
20 to 30, with light	758523	1935	200	1	26	89	92	92							
	761849	1936	200	0	37	84	92	92	92	94	95	99			
	766403	1937	100	9	55	83	93								
	766101	1937	200	1	34	84	91	94							
	769357	1938	100	0	7	85	86	89	90	90	91	94	95	97	97
Total or mean			1,100	2	32	85	91	92	92	93	93	95			
20 to 30, light excluded	761849	1936	200	0	3	4	17	21	25	26	28	45	51		
Room to 35	758523	1935	200	0	35	81	91	94							
	761849	1936	200	2	65	87	90	92	96						
Total or mean			400	1	50	84	90	93	95						
15 to 25	761849	1936	200	0	0	0	0	0	1	2	2	20	22	23	
	766403	1937	100	0	5	7	7	21	21	21					
	766101	1937	200	0	0	0	3	21	21	22	22				
Total or mean			500	0	2	2	3	14	14	15	17	21			

STORED SEED

The course of germination at 20° to 30° C. with light in successive tests of sample 758523, collected September 23, 1935, and tested 1 day, 2 months, 3½ months, and 1 year after harvest, is shown in figure 9. In 1938 sample 769357 was placed to germinate on the day it was harvested and after 8 intervals of approximately 1 month each. A summary of the complete results is presented in table 5, and the course of germination for some of the tests at 20° to 30° C. with light is shown

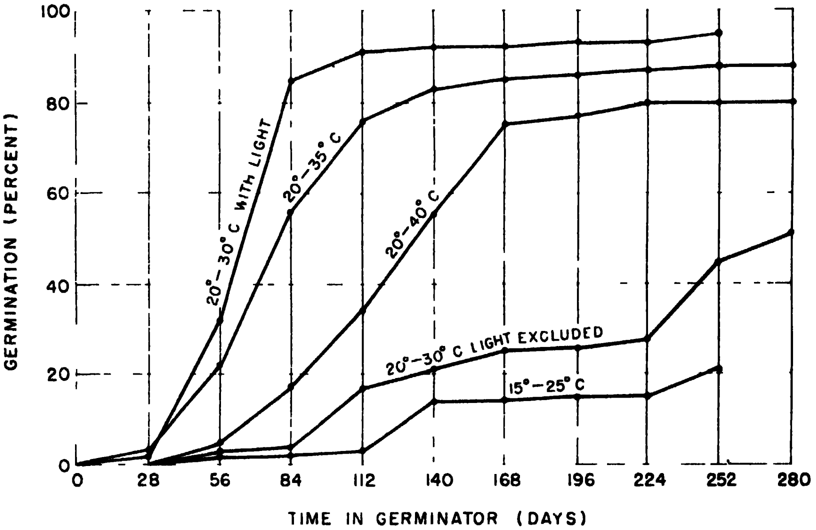

FIGURE 8. Mean germination of fresh seed of *Digitaria sanguinalis* for all years, showing the progress of germination at the various temperature alternations.

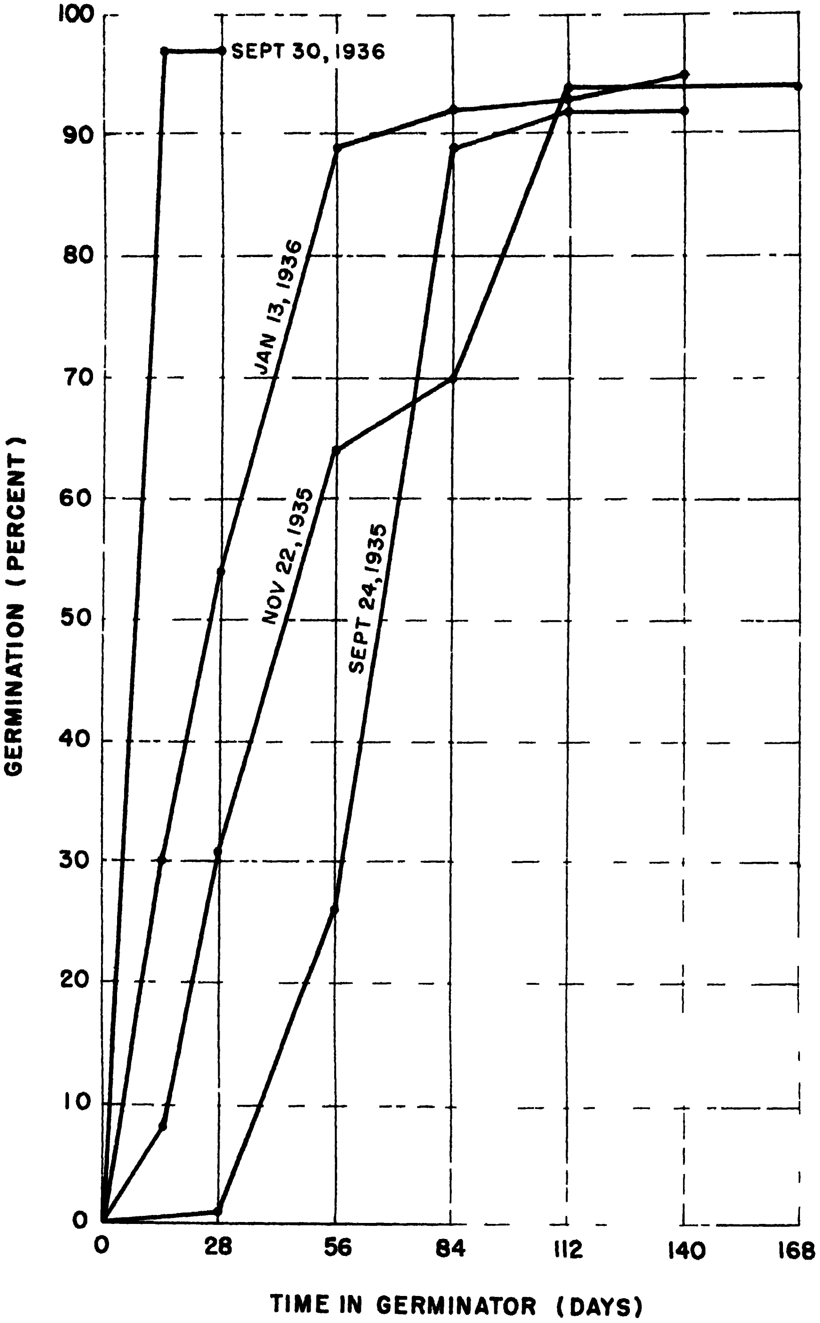

FIGURE 9.—Progress of germination of seed of *Digitaria sanguinalis* at 20° to 30° C. alternation with light, at different dates after harvesting. Sample 758523, collected September 23, 1935.

TABLE 5.—*Summary of germination at different temperatures of seed of Digitaria sanguinalis when tested at successive periods after harvest*

[Sample 769357, collected Sept. 8, 1938. 100 seeds used for each test]

Temperature alternation (° C.) and light condition	Period of germination count	Germination[1] in tests started Sept. 8, 1938	Oct. 6, 1938	Nov. 3, 1938	Dec. 1, 1938	Jan. 5, 1939	Feb. 2, 1939	Mar. 2, 1939	Mar. 31, 1939	Apr. 28, 1939
	Days	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
20 to 40	14	0	0	0	[illegible]	30	42	10	72	70
	28	0	1	2	9	35	46	43	71	71
	56	2	3	1	10	39	50	44	76	74
	84	11	8	6	11	41	52	45	77	76
	112	33	39	28	35	45	54	51	78	77
	140	54	64	59	53	48	63	61	81(19)	
	168	61	76	72	58	58	75	68(30)		
	196	65	80	75	60	68	77(21)			
	224	73	80	75	65	70				
	252	71	80	77	66					
	280	71	81	78						
	308	74	81							
	336	7								
20 to 35	14	0	0	2	27	36	61	78	86	90
	28	0	0	11	36	51	73	84	91	92
	56	14	3	39	47	63	79	85	93	94
	84	55	57	74	60	68	80	87	93	94
	112	76	78	75	71	73	83	88	93	94( )
	140	84	86	80	78	78	84	90	93(6)	
	168	86	87	84	81	80	88	91(8)		
	196	86	90	86	81	86	88(10)			
	224	88	92	86	85	87(9)				
	252	91	92	89	85(12)					
	280	91	92	90(8)						
	308	93	93( )							
	336	93(1)								
20 to 30 without definite light exposure	14	0	0	1	16	29	58	55	62	85
	28	0	1	31	45	52	74	75	81	87
	56	21	38	63	62	64	84	80	84	90
	84	54	58	62	68	65	87	87	85	91
	112	75	63	75	70	66	89	87	86	91(9)
	140	77	66	75	71	72	90	88	88(12)	
	168	79	66	76	74	75	91	88(10)		
	196	79	67	79	77	77	91(8)			
	224	79	71	80	81	78(20)				
	252	[illegible]	73	84	[illegible](13)					
	280	87	81	[illegible](11)						
	308	91	81(11)							
	336	92(4)								
20 to 30 with light	14	0	0	0	6	29	33	18	71	93
	28	0	2	16	38	51	71	78	90	96
	56	[illegible]	65	75	67	82	87	90	95	97
	84	85	72	88	71	86	93	95	96	98
	112	86	81	89	83	90	95	95	97	99(0)
	140	89	85	91	84	93	95	96	97(1)	
	168	90	8[illegible]	91	90	93	95	97(1)		
	196	90	86	93	91	93	97(1)			
	224	91	93	9[illegible]	93	97(1)				
	252	94	95	97	95(3)					
	280	9[illegible]	96	98						
	308	97	97(2)							
	336	97(2)								

[1] Figures in parentheses after final percentage show percentage of apparently sound seed remaining when test was discontinued.

[2] Many apparently sound seed remaining when test was discontinued.

graphically in figure 10. With both of these samples the germination in 28 days was progressively greater as the seed aged, but the germination finally obtained was about the same, whatever the age of the seed. Seed of sample 769357 (table 5) placed to germinate on April 28, about $6\frac{3}{4}$ months after collection, showed about the same germination in 28 days as the freshly harvested seed in 252 days. The change in rate of germination is also shown in the part of figure 14 that presents tests without prechilling. The relation of time since harvest to germination is brought out more clearly by a retabulation of the germination results at 20° to 30° C. with light on the basis of the actual date of the germination count (table 6). Germination on May 25 was essentially the same whether the seeds had been moist in the germinator all the time since the seed was harvested, or had been stored dry for different portions of the time. Also, germination was practically completed (95 to 99 percent) by August 17 irrespective of the date that germination started.

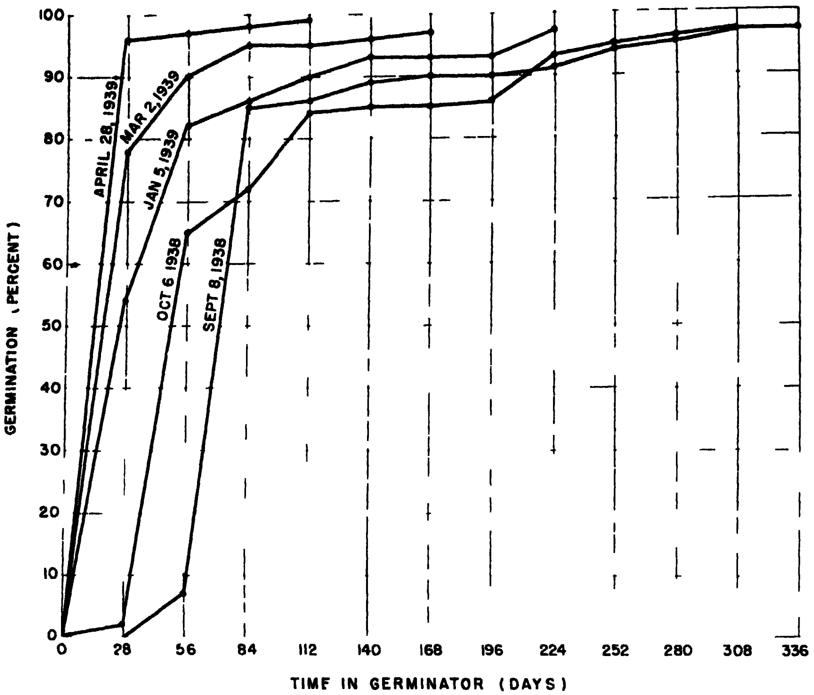

FIGURE 10.—Progress of germination of seed of *Digitaria sanguinalis* at 20° to 30° C. alternation with light, tested at successive dates after harvesting. Sample 769357, collected September 8, 1938.

TABLE 6.—*Germination of seed of Digitaria sanguinalis when tested at successive periods after harvest, at 20° to 30° C. alternation with light, based on the actual date of germination count*

[Sample 769357 collected Sept. 8, 1938]

Date of germination count	Germination in tests started—								
	Sept. 8	Oct. 6	Nov. 3	Dec. 1	Jan. 5	Feb. 2	Mar. 2	Mar. 31	Apr. 28
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
May 25	95	94	94	90	93	95	95	95	96
June 25	96	96	[illegible]	92	93	[illegible]	95	96	97
July 20	97	97	97	94	93	[illegible]	96	97	98
Aug. 17	98	97	98	95	97	97	97	97	99

After approximately 8 months' storage, the differences in rate of germination at the different temperature alternations (fig. 11) had largely disappeared. A great part of the total germination at each

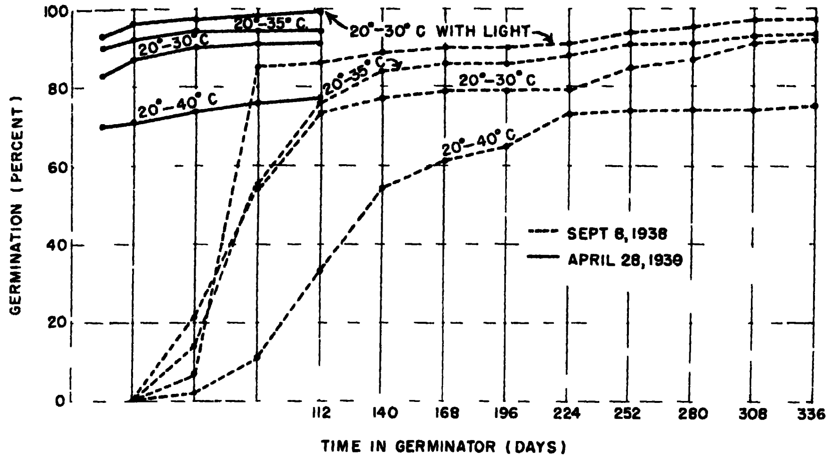

FIGURE 11.—Progress of germination of seed of *Digitaria sanguinalis* at different temperature alternations, tests at harvesting and after storage for 8 months. Sample 769357, collected September 8, 1938.

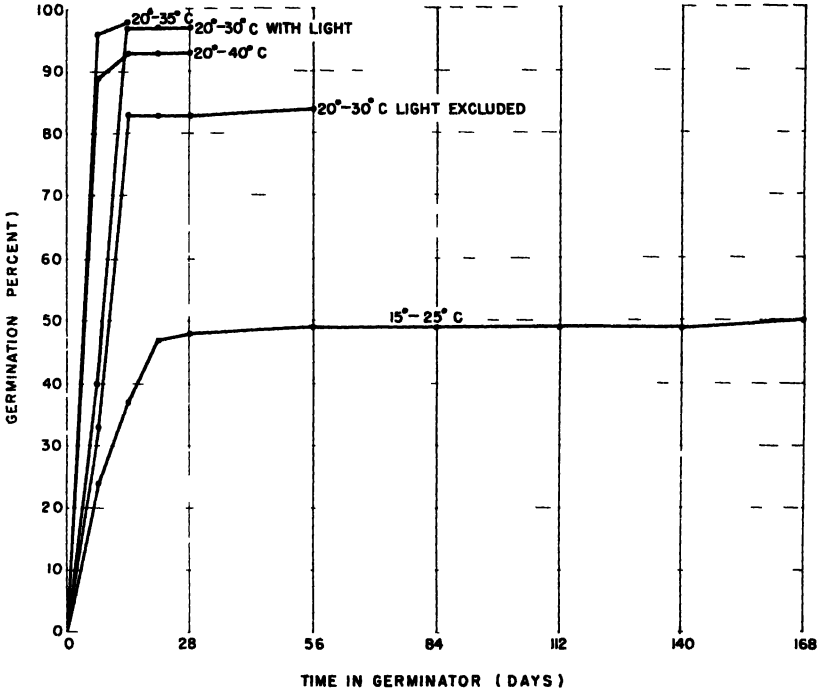

FIGURE 12.—Progress of germination of seed of *Digitaria sanguinalis* at different temperature alternations after storage for 1 year. Sample 758523.

alternation was in the first 14 days. However, the total germination at 20° to 40° C. was distinctly lower than at the other alternations. Germination of seed after 1 year of storage (sample 758523, fig. 12) was more than 90 percent and was practically completed in 14 days.

at 20° to 40° as well as at 20° to 30° with light, and at 20° to 35°. At 15° to 25° (sample 758523, fig. 13), the 1-year-old seed germinated less than 50 percent and the 2-year-old seed less than 80 percent in 56 days. It was not until the seed had been held in dry storage for 3 years that germination was fairly prompt and complete at this temperature, although at the other germination temperatures used there was no further change in response after the first year.

PRECHILLED SEED

The results of germination of the seed of *Digitaria sanguinalis* after prechilling were erratic. Sample 758523, collected in 1935, showed essentially the same percentage of germination after prechilling for 1 and for 2 weeks as without such treatment. When the seed was 2 months old, prechilling for 2 weeks brought about a distinct hastening of germination, but treatment for 4, 8, and 12 weeks caused distinct injury, resulting in progressively lower percentages of final germination as the period of chilling was lengthened.

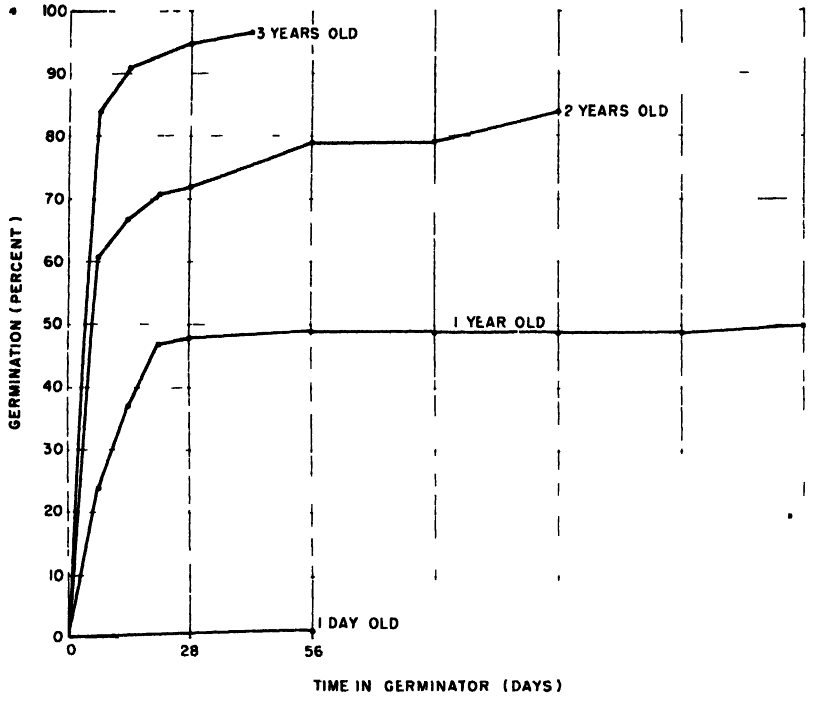

FIGURE 13.—Change of germination response at 15° to 25° C. of seed of *Digitaria sanguinalis* stored over a period of 3 years with tests at yearly intervals. Sample 758523.

The results of prechilling on sample 761849, collected in 1936, are presented in table 7. Prechilling the freshly harvested seed for 2 and for 4 weeks caused a small but definite increase in early germination, but there was a distinct tendency for the later germination to fall below that of the control. The older seed was definitely stimulated by prechilling for 2 and 4 weeks. In both instances there was definite injury from treatments longer than 4 weeks.

TABLE 7.—*Effect of prechilling at 3° C. on germination at 20° to 35° alternation of seed of Digitaria sanguinalis when tested immediately after harvest and about 2 months later*

[Sample 761849 collected Sept. 28, 1936. 200 seeds used in each test]

Period of germination count (days)	Germination after prechilling for indicated number of weeks in tests started Sept. 30, 1936				Germination after prechilling for indicated number of weeks in tests started Dec. 9, 1936			
	0	2	4	8	0	2	4	6
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
28	0	1			78	89		
56	29	47	70		80	92	92	84
84	72	75	79	69	83	93	92	85
112	84	79	81	70	86	95	92	
140	89	84	84	71	86	93	93	
168	91	85	85	71	86		94	
196	91	85	85	71	89		94	
224	91	85	85	72	91		94	
252	91	86	86					
280		88	87					
308		90	89					

The results of prechilling seed collected in 1938 (sample 769357) at monthly intervals after harvest are given in table 8. The relative increase in the early germination caused by prechilling for 4 weeks is shown in figure 14, based on germination in 56 days (tables 5 and 8).

TABLE 8.—*Summary of germination of seed of Digitaria sanguinalis, after prechilling at 3° for 4 weeks, when tested at successive periods after harvest*

[Sample 769357 collected Sept. 8, 1938. 100 seeds used in each test]

Temperature alternation (° C.)	Time of germination count	Germination in tests started Sept. 8, 1938	Oct. 6, 1938	Nov. 3, 1938	Dec. 1, 1938	Jan. 5, 1939	Feb. 2, 1939	Mar. 2, 1939	Mar. 31, 1939	Apr. 28, 1939
	Days	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
20 to 30	28	[illegible]	11	12	[illegible]	72	68	72	77	73
	42	18	36	[illegible]	82	90	85	86	92	92
	56	[illegible]	[illegible]	86	89	92	91	93	95	91
	84	[illegible]	[illegible]	89	90	92	96	94	96	96
	112	[illegible]	[illegible]	89	90	93	96	94	96	96(2)
	140	82	[illegible]	89	90	92	96	94	96(2)	
	168	82	[illegible]	93	91	92	96	94(1)		
	196	83	89	[illegible]	91	92	96(2)			
	224	85	94	94	92	92(5)				
	252	86	[illegible]	[illegible]	92(8)					
	280	87	90	[illegible](2)						
	308	88	90(3)							
	336	88								
	343	89(1)								
20 to 35	28	9	34	71	69	80	[illegible]	87	95	82
	42	33	63	92	95	93	96	95	96	96
	56	62	89	91	97	96	97	98	98	97
	84	79	[illegible]	94	98(0)	96	99	98	98(0)	98(0)
	112	82	95	95		96	99	98		
	140	85	96	95		97	99	98		
	168	86	96	95		97	99	98(0)		
	196	87	97	95		97	99(0)			
	224	89	97	95		96(1)				
	252	90	96(0)	95(0)						
	280	90								
	308	90								
	336	90								
	343	90(3)								

[1] Figures in parentheses after final percentage show percentage of apparently sound seed remaining when test was discontinued.

By the time the seed was 8 weeks old germination of the seed prechilled 4 weeks was above 90 percent after 28 additional days at 20° to 35° C., whereas germination of the unchilled seed was about 40 percent. The test, started 7 months after harvest, was the first in which seed without prechilling germinated above 90 percent in 56 days. The results in table 8 show that germination after prechilling was quicker

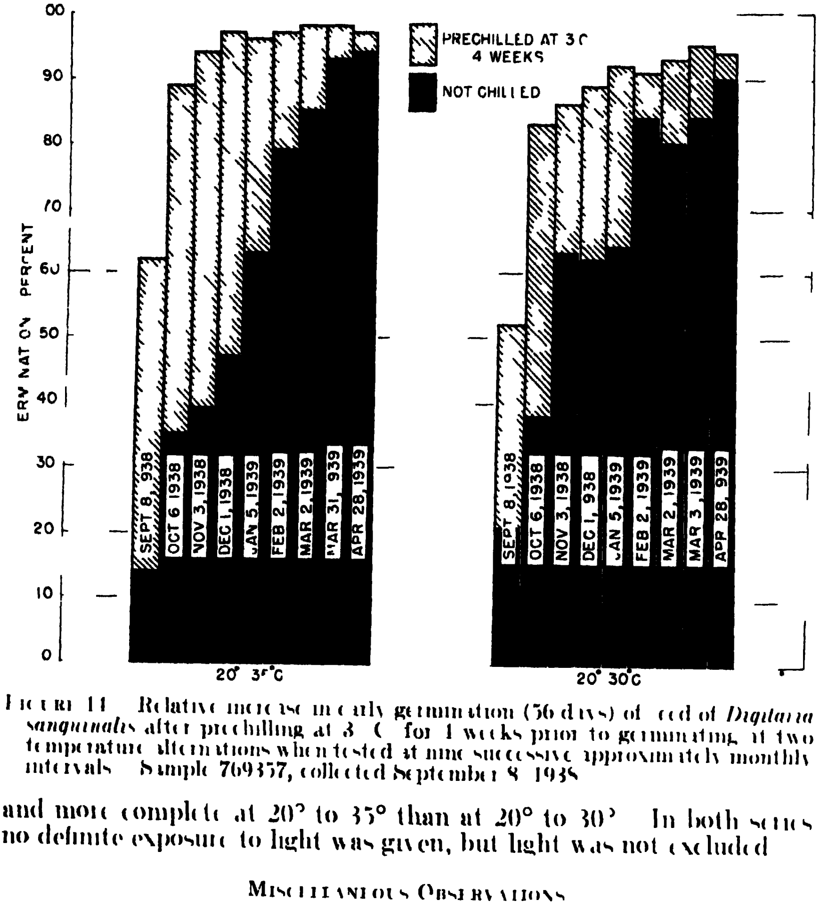

FIGURE 11.—Relative increase in early germination (56 days) of seed of *Digitaria sanguinalis* after prechilling at 3° C. for 4 weeks prior to germinating at two temperature alternations when tested at nine successive approximately monthly intervals. Sample 769357, collected September 8, 1938.

and more complete at 20° to 35° than at 20° to 30°. In both series no definite exposure to light was given, but light was not excluded.

MISCELLANEOUS OBSERVATIONS

In the germination tests of *Digitaria sanguinalis* discussed above the substratum was moistened with water. In most instances comparable tests were made with the substratum moistened with a 0.2-percent solution of potassium nitrate. The effect of potassium nitrate solution was variable, no definite or consistent effect was observed except a stimulation of mold development even greater than with *D. ischaemum*.

In contrast to the result with *Digitaria ischaemum* there was a definitely beneficial effect of light on the germination of seed of *D. sanguinalis*. The results in table 4 and figure 8 for freshly harvested seed show that germination of sample 761849 was much faster and better at 20° to 30° C. with light than at the same temperature with light excluded by enclosing the tests in a tin box. Table 4 also shows faster germination of samples 758523 and 761849 at room temperature to 35° than at 20° to 35°; in this comparison the temperatures for the longer period of the alternation, although similar, are not identical.

The effect of exclusion of light when the seed was tested approximately 3½ months after harvest is shown in figure 15. The benefit of light is marked at 20° to 30° C., but at room temperature to 35° the difference in germination between light and dark is not so great. When this same sample was tested 1 year after harvest (fig. 12) about 80 percent of the seeds germinated equally well in the light or in the dark, but the remainder of the seeds germinated well in the light and very slowly if at all in the dark. The results with sample 769357 tested at intervals after harvest (table 5) show rather consistently an earlier start of germination at 20° to 30° without definite light exposure

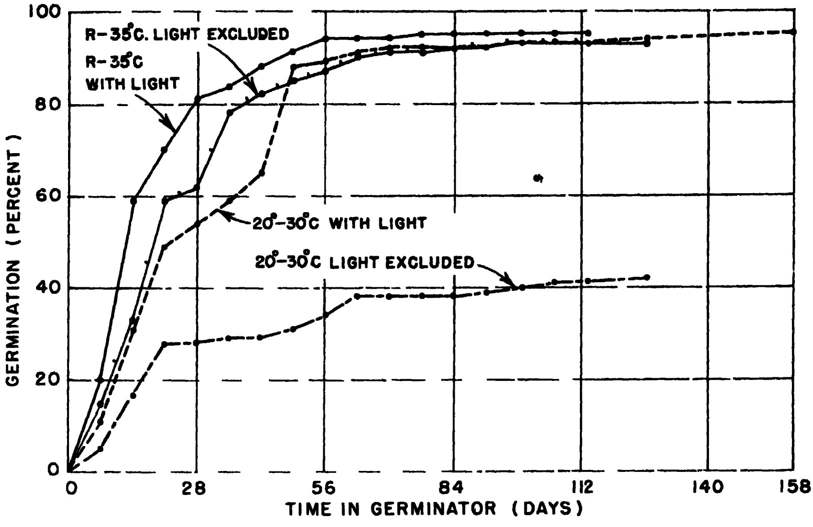

FIGURE 15.—Progress of germination of seed of *Digitaria sanguinalis* at two temperature alternations, with light and with light excluded. Sample 758523, collected September 23, 1935, tests started January 13, 1936.

(except some light from electric lamps used to heat the chambers and some light admitted during transfer of tests and during counting) than at 20° to 30° with light. However, with a definite light exposure, later germination was more rapid and final germination percentages usually were higher. These two series of tests were in entirely different chambers, and differences in humidity and variables other than light may have influenced the results to some extent.

Treatments of seed of one sample with concentrated sulfuric acid for various periods from ½ minute to 3 minutes brought about an earlier start of germination than that which took place without acid treatment.

DISCUSSION

Probably the most striking feature of the germination of these two species of *Digitaria* is the extremely slow but steady germination of the freshly harvested seed under the most favorable conditions. In general more than 100 days was required for 80 percent of the sample to germinate, and 196 days would seem to be the shortest time in which one could expect completion of germination. In contrast with this extremely slow germination of seed freshly harvested, seed that was 1 year old germinated rather rapidly, germination being completed in 7 to 14 days.

Both species of *Digitaria* germinated very little at the various constant temperatures tried and seemed to require daily temperature alternations. Liebenberg (*11*) in 1884 appears to have been the first to call attention to the value of definite daily alternations of temperature in seed germination. Since then many have shown the need of alternating temperatures for germination of many kinds of seeds.

With freshly harvested seed there was a distinct difference in the response of the two species to the different temperature conditions used. With seed of *Digitaria ischaemum* differences in the length of incubation time before germination started were a striking feature in the response to the temperature alternations 20° to 40° C., 20° to 35°, and 20° to 30°. On the other hand, seed of *D. sanguinalis* did not show much difference in the incubation period, but the rate of germination was markedly affected by germination temperature. The alternation 20° to 40° was distinctly favorable for a large proportion of the seeds of each sample of *D. ischaemum;* but at this alternation seeds of *D. sanguinalis* had a long incubation period, a slow rate of germination, and a low total germination. The alternation 15° to 25° was distinctly unfavorable for both species, but especially so for *D. sanguinalis*. The definitely beneficial effect of light exposure for the germination of *D. sanguinalis* affected the results of the temperature studies with this species. It would appear that the alternations 20° to 35° and 20° to 30° were equally good, but that light was necessary for full germination at either temperature condition. The small amount of light from the lamps used to heat the chambers greatly increased germination over that obtained when light was totally excluded, but it was not sufficient for rapid and complete germination. It is possible that the very low results at 15° to 25° with *D. sanguinalis* were due in part to the small amount of light in the chambers at these lower temperatures.

The beneficial effect of dilute solutions of nitrates usually has been associated with the beneficial effect of light, although many exceptions have been noted in the literature. No explanation is offered for the retarding influence of potassium nitrate on germination of *Digitaria ischaemum* at higher temperatures and the stimulation at lower temperatures, nor for the apparent lack of consistent influence of potassium nitrate on the germination of *D. sanguinalis* although this species is benefited by light exposure.

It has been recognized generally that the seeds of a sample may not respond alike to germination conditions. This fact is illustrated by the present results, especially by the favorable response of a large proportion of the fresh seeds of *Digitaria ischaemum* to the 20° to 40° C. alternation and the decidedly unfavorable response of a small proportion to this temperature condition. This small portion gave a slightly more favorable response to the 20° to 35° alternation. At 20° to 30° the rate of germination began to fall after about one-half of the seeds had germinated. Aside from the long period of incubation before the start of germination, the most striking feature in the progress of germination of these two species is perhaps the variable proportion of resistant seeds at the different temperature conditions.

Haasis (*6*), Edwards (*5*), and Livingston and Haasis (*12*) have suggested that a given lot of seeds might be separated into physiological classes on the basis of differences in rate of germination. The results presented in this paper indicate that the proportions in the different classes would vary greatly with the temperature conditions of germination.

Both species of *Digitaria* showed a marked change in the progress of germination as the seed aged. This change was evident first as a shortening of the incubation period before germination, and later as a marked increase in the rate of germination. Even at the most favorable temperature condition for each species, a certain proportion of the freshly harvested seed (20 to 40 percent) was very resistant to germination, but as the seed aged this proportion became smaller until at about 8 months after harvest only 5 to 10 percent were resistant. Although seed of *D. sanguinalis* 1 to 3 months old started to germinate much more quickly than fresh seed, there was a suggestion that a small proportion of this slightly older seed was more resistant to germination than the fresh seed, as is shown in figure 10 and table 5.

With many kinds of seeds that germinate readily, the germination response to temperature may be similar to the growth response of the seedling, as would seem to be the case in the results presented by Haasis (*6*) and by Edwards (*5*). On the other hand, many kinds of seeds require special conditions for the initiation of the germination process that may have no close relation to the growth response of the seedling. The difference in the progress of germination at the various temperature conditions of fresh seed of both species of *Digitaria* would indicate that a major effect of germination condition was in controlling preliminary changes in the seed necessary for the initiation of germination. However, the two species responded very differently in this respect to the 20° to 40° C. alternation. As the seed aged it became less sensitive to conditions controlling initiation of germination, so that by the time the seed was a year old germination started promptly under all the temperature conditions used. At this time the small difference in response to temperature observed in the germination of *D. ischaemum* was due to differences in growth rate. The seed of *D. sanguinalis* had mostly afterripened in 1 year when measured by its response to 20° to 40°, 20° to 35°, and 20° to 30° alternations, with light; but at 15° to 25° it was not until the seed was 3 years old that the rate and completeness of germination equaled 1-year-old seed of *D. ischaemum*. A small percentage of the seeds of *D. sanguinalis* required light for germination even after 1 and 2 years' storage.

Prechilling the moist seed at 3° C. brings about a quicker initiation of the germination process and a faster rate of germination. Apparently the preliminary changes that take place slowly in dry stored seed or in seed held under germination conditions are brought about much more quickly in seed held moist at a low temperature. Fresh seed of *Digitaria ischaemum* prechilled for 8 weeks germinated at 20° to 35° almost as promptly as 1-year-old seed without chilling, and the progress of germination of 1-month-old seed after prechilling for 2 weeks was similar to that of 6-month-old seed that had not been prechilled. With fresh seed of *D. ischaemum* a short period of chilling caused a few seeds to germinate promptly, but the remaining seeds germinated about as slowly as untreated seed and there was some indication that they were slower (fig. 3). As the seed became older or with a longer period of prechilling of fresh seed the proportion of seeds resistant to germination was smaller. Seed stored dry for 1 month and then prechilled for 4 weeks germinated very much like untreated seed that had been stored dry for 6 months longer. The variation in the condition of individual seeds of a sample is indicated by the response to different periods of chilling as well as by the response to different temperature conditions.

The germination results after prechilling were much more definite with seed of *Digitaria ischaemum* than with seed of *D. sanguinalis*. The results with the latter species were affected by a definite tendency for injury to the seeds to occur during long periods of prechilling. It is possible that this injury is related to immaturity of some of the seeds and to the presence of fungi. With the seed of *D. sanguinalis* used and under the conditions of these experiments periods longer than 4 weeks at a low temperature caused injury, and this period of treatment was not long enough to overcome the dormancy of all seeds when tested immediately after harvest.

Davis and Rose (*3*) have shown that prechilling *Crataegus* seed for 2 to 3 months brings about changes in the dormant embryo that initiate normal germination. Toole (*14*) and Whitcomb (*16*) have shown that freshly harvested cereals in which resistance to germination at higher temperatures is caused by the coats will germinate readily at higher temperatures after being prechilled for 5 to 7 days. The response of the seed of *Digitaria* to acid treatment as well as the response to chilling would indicate that the slow germination of fresh seed of these species probably is due both to coat restrictions and to embryo dormancy.

No study of seed structure was made, but Harz (*9*) states that the coat structure of *Digitaria sanguinalis* is similar to that of *Panicum miliaceum*. The latter species is not especially resistant to germination.

In comparing the response of seeds to germination conditions the general progress of germination would seem to be a better guide than the germination attained after any time interval. The optimum condition for germination may, as here, involve many factors, but it would seem to be best expressed as the condition at which germination progresses at the fastest rate and at which germination of the viable seeds is completed in the shortest time.

Although the seeds of these two species of *Digitaria* show some differences in their response to temperature, light, and dilute salt solution, they are alike in requiring certain changes in the coats or in the embryo, or in both, before germination starts. These changes take place very slowly in dry storage or when the seed is moist at temperatures suitable for germination. Such changes are affected to some extent by different germination conditions, but take place most rapidly in the moist seed at low temperatures at which germination does not occur.

SUMMARY

The progress of germination of seed of the two crabgrasses *Digitaria ischaemum* and *D. sanguinalis* was determined under different conditions of temperature when freshly harvested and after various periods of storage. The effect of prechilling the moist seed at 3° C. for various periods, as well as the effects of light and of dilute solutions of potassium nitrate, were studied.

Germination was more or less complete at the four temperature alternations used, 20° to 40° C., 20° to 35°, 20° to 30°, and 15° to 25°.

Fresh seed of both species required a preliminary incubation period of 28 to 56 days before germination started; at least 196 days was required for completion of germination.

As the seed aged in dry storage, the preliminary period was shortened and the rate of germination increased. With 1-year-old seed, germination started promptly and was completed in 14 days or less.

Prechilling the fresh seed of both species hastened the rate and the completion of germination. Prechilling for 8 weeks was necessary for prompt germination of fresh seed, but the time required for prechilling decreased as the seed aged.

In a given sample, some seeds were affected by germination treatment differently from others, and the proportion of the seeds stimulated or retarded varied with the treatment.

Freshly harvested seed of *Digitaria ischaemum* had the shortest incubation period at the 20° to 40° C. alternation, but a small proportion of the seeds was slower in germinating at this temperature than at 20° to 35°. At 20° to 30° the incubation period was longer and a larger proportion of the sample was resistant to germination. At 15° to 25° germination started at about the same time as at 20° to 30°, but the rate of germination was slower and with some samples germination was not completed during the period of observation.

Freshly harvested seed of *Digitaria ischaemum* germinated promptly and completely at 20° to 35° C. alternation after 8 weeks' prechilling, but after 2 and 4 weeks' prechilling a portion of the sample was slow to germinate. As the seed aged a shorter period of treatment was sufficient for prompt and complete germination.

Germination of freshly harvested seed of *Digitaria sanguinalis* was essentially the same at 20° to 30° C. and at 20° to 35° alternations, but proceeded at a slower rate at 20° to 40°. At 15° to 25° germination was very slow and incomplete.

The seed of *Digitaria sanguinalis* used was injured by long periods of prechilling, so that maximum germination of fresh seed was not obtained after prechilling. After 1 month of dry storage, prechilling for 4 weeks was followed by fairly prompt and complete germination at 20° to 35° C. alternation; and after 3 or 4 months of dry storage prechilling for 4 weeks resulted in about the same rate of germination as was obtained with 1-year-old seed without chilling.

Treatment of the seed of both species with concentrated sulfuric acid reduced the incubation period of fresh seed, but not as much as an optimum period of prechilling.

Moistening the substratum with 0.2-percent solution of potassium nitrate definitely hastened germination of seed of *Digitaria ischaemum* at the 15° to 25° C. and 20° to 30° alternations, and definitely retarded it at 20° to 35° and 20° to 40°, but had no definite effect on the seed of *D. sanguinalis*.

Total exclusion of light had no definite effect on germination of *Digitaria ischaemum*, but greatly retarded germination of *D. sanguinalis* at 20° to 30° C. alternation and slightly retarded it at 20° to 35°.

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INTERSPECIFIC GENETIC RELATIONSHIPS IN LACTUCA[1]

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INTRODUCTION

For a number of years the United States Department of Agriculture has conducted an extensive lettuce-breeding program. The work has been divided between the United States Horticultural Station at Beltsville, Md., and the United States Horticultural Field Station at La Jolla, Calif.

Since 1935, investigations have been conducted at the Beltsvillle station for the purpose of locating genes for resistance to the aster yellows virus in lettuce. In these tests have been included most of the varieties listed by American seedsmen, as well as many strains received from foreign sources through the Division of Plant Exploration and Introduction. Many varieties of the cultivated species *Lactuca sativa* L. have been grown to determine their resistance or susceptibility to this virus. Since no evidence of resistance was found in any of the varieties tested, an extensive study was made of wild species within the genus *Lactuca*. Some degree of resistance to aster yellows virus has been found in certain of the wild species in tests not yet reported.

For several years workers at the La Jolla station have been studying some of these wild species in breeding for resistance to various diseases and for types better adapted to specific localities.

The question of the genetic compatibility of the various species has naturally arisen. Difficulty in hybridizing some of the wild species with *Lactuca sativa* was expected, owing to the variation in the chromosome number within the genus. According to Babcock, Stebbins, and Jenkins (*1*),[3] the genus *Lactuca* includes species having 8, 9, and 17 pairs of chromosomes.

Little is known of the genetic compatibility of the species of *Lactuca*. Whitaker and Jagger (*9*) have reported on hybridization experiments confined largely to crosses of *L. sativa* with other species. Of nine interspecific crosses attempted, only two, *L. sativa* × *L. Serriola* (*L. scariola*)[4] and *L. graminifolia* × *L. canadensis*, resulted in fertile seed. In both of these the hybrids proved to be fertile.

This paper is a joint report of the results obtained in interspecific hybridization in *Lactuca* at the stations at Beltsville and La Jolla. In the investigations herein reported, numerous interspecific crosses not previously reported were made, with results that cast some light upon the relationship of certain species within the genus.

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[2] The writers are indebted to the Division of Plant Exploration and Introduction, Bureau of Plant Industry, for supplying much of the species material used in the investigations herein reported. Thanks are extended to G. L. Stebbins, Jr., Division of Genetics, University of California, for material supplied and for identifying some of the species, and also to S. F. Blake, of the Division of Plant Exploration and Introduction, for assistance in identifying some of the species.

[3] Italic numbers in parentheses refer to Literature Cited, p. 107.

[4] The writers have been informed by Dr. S. F. Blake, of the Division of Plant Exploration and Introduction, Bureau of Plant Industry, that the species generally called *Lactuca scariola* L. should be listed as *L. serriola* L. on the evidence that Linnaeus' first classification used the latter name. This suggested change in the name of the species is supported by Dr. G. L. Stebbins, Jr., of the Department of Genetics, University of California.

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MATERIALS AND METHODS

The materials used in the interspecific hybridization studies made by the writers were obtained from the following sources:

Lactuca altaica Fisch. and Mey., from Egypt, through Division of Plant Exploration and Introduction.

L. sativa L., from private stocks.

L. serriola L., collected in the District of Columbia and Maryland.

L. serriola var. *integrata* (Gren. and Godr.) Farwell, collected in the District of Columbia and Maryland.

L. indica L., from China, through G. L. Stebbins, Jr.

L. indica L., from China, through Division of Plant Exploration and Introduction.

L. laciniata (Houtt.) Makino, from Asia, through Division of Plant Exploration and Introduction.

L. tatarica (L.) C. A. Mey., from Cap d'Antibes, France, through G. L. Stebbins, Jr.

L. raddeana Maxim., from Union of Socialist Soviet Republics, through Division of Plant Exploration and Introduction

L. virosa L., from Amsterdam, the Netherlands, through Division of Plant Exploration and Introduction.

L. virosa L., from Surrey, England, through Division of Plant Exploration and Introduction.

L. virosa L., from Stockholm, Sweden, through Division of Plant Exploration and Introduction.

L. canadensis L., collected in Maryland.

L. graminifolia Michx., from South Carolina, through J. B. Norton.

L. floridana (L.) Gaertn., collected in Maryland.

L. saligna L., from Ohio, through G. L. Stebbins, Jr.

L. spicata (Lam.) Hitchc., collected in Maryland, Virginia, and Pennsylvania

L. perennis L., from Wayside Gardens, Mentor, Ohio.

L. bourgaei (Boiss.) Irish and Taylor, from England, through Division of Plant Exploration and Introduction.

L. cretica Desf., from Africa, through G. L. Stebbins, Jr.

L. chondrillaeflora Bor., from France, through Division of Plant Exploration and Introduction.

In the work at Beltsville, all of the plants used for crossing purposes were grown in 10 inch clay pots in a closely screened greenhouse. At La Jolla the plants were grown outside and the flowers were covered after pollination to prevent contamination.

Some difficulty was met in bringing the different species into flowering at the same time. Some of the species are annual and some biennial or perennial. Species that were found to be easily grown were planted at successive dates so as to have the plants in flower almost continuously.

In making the crosses, the commonly used technique of pollen removal by water, described by Oliver (*6*), was employed.

Diseases and insects caused difficulty in growing the numerous species under the same environment. Some species are very susceptible to disease when grown under glass. *Lactuca canadensis* is susceptible to mildew when grown during the winter months, and several species of the *serriola* group are susceptible to crown rot during cloudy, damp weather. *L. laciniata* is very susceptible to injury by red spider and a number of foliage diseases.

RESULTS

The results of the interspecific cross-pollinations are summarized in table 1.

TABLE 1.—*Summary of data on interspecific hybridization in Lactuca*[1]

Species and number of chromosomes	At Beltsville, Md.				At La Jolla, Calif.			
	Results of cross		Fertility of F_1		Results of cross		Fertility of F_1	
	Cross as indicated	Reciprocal	Cross as indicated	Reciprocal	Cross as indicated	Reciprocal	Cross as indicated	Reciprocal
L. sativa (9) ×								
L. altaica (9)	H	H	F	F				
L. serriola (9)	H	H	F	F	H	H	F	F
L. saligna (9)	O	O			X	X		
L. indica (9)	O	O			X	X		
L. tatarica (9)	O	O			O	O		
L. laciniata (9)	O	O			O	O		
L. raddeana (9)	O	O			X	X		
L. virosa (9)	O	O			H		S	
L. perennis (9)	O	X			O	O		
L. bourgaei (8)	O	O			X	X		
L. cretica (8)	O	X			O	O		
L. spicata (17)	O	O			X	X		
L. canadensis (17)	O	O			O	O		
L. graminifolia (17)	O	O			O	O		
L. floridana (17)	O	O			O	O		
L. chondrillaeflora (9)	X	X			O	O		
L. serriola (9) ×								
L. indica (9)	O	X			X	X		
L. tatarica (9)	O	O			X	X		
L. laciniata (9)	O	O			X	X		
L. raddeana (9)	O	X			X	X		
L. perennis (9)	O	X			X	X		
L. virosa (9)	O	O			X	X		
L. bourgaei (8)	O	X			X	X		
L. canadensis (17)	X	O			X	X		
L. graminifolia (17)	O	O			X	X		
L. floridana (17)	O	O			X	X		
L. virosa (9) ×								
L. cretica (8)	O	X			O	X		
L. chondrillaeflora (9)	X	X			O	O		
L. tatarica (9)	O	O			X	X		
L. indica (9)	O	O			X	X		
L. laciniata (9)	O	X			O	O		
L. saligna (9)	O	O			X	X		
L. raddeana (9)	O	O			X	X		
L. perennis (9)	O	O			O	X		
L. graminifolia (17)	O	O			X	H		PF
L. floridana (17)	O	X			X	X		
L. canadensis (17)	O	O			O	O		
L. spicata (17)	O	X			X	X		
L. saligna (9) ×								
L. serriola (9)	H	O	PF		X	X		
L. indica (9)	O	X			X	X		
L. tatarica (9)	O	O			X	X		
L. laciniata (9)	O	X			X	X		
L. raddeana (9)	O	X			X	X		
L. canadensis (17)	O	X			X	X		
L. floridana (17)	O	X			X	X		
L. graminifolia (17)	O	O			X	X		
L. tatarica (9) ×								
L. laciniata (9)	H	H			X	X		
L. indica (9)	H	H	S	S	X	X		
L. perennis (9)	O	O			X	X		
L. floridana (17)	H	X	S		X	X		
L. muralis (9)	O	O			X	X		
L. raddeana (9)	O	H		S	X	X		
L. laciniata (9) ×								
L. indica (9)	H	H	F	F	X	X		
L. floridana (17)	H	H	S	S	X	X		
L. chondrillaeflora (9)	X	X			O	O		

[1] Key to letter symbols:

F the hybrids obtained were self fertile.
X the cross was not attempted.
O cross was attempted but failed.
PF the hybrids were only partly fertile.
S the hybrids obtained were self sterile.
H hybrid seedlings were obtained.
H hybrids were obtained but fertility is not yet known.

TABLE 1.—*Summary of data on interspecific hybridization in Lactuca*—Continued

Species and number of chromosomes	At Beltsville, Md.: Results of cross: Cross as indicated	At Beltsville, Md.: Results of cross: Reciprocal	At Beltsville, Md.: Fertility of F_1: Cross as indicated	At Beltsville, Md.: Fertility of F_1: Reciprocal	At La Jolla, Calif.: Results of cross: Cross as indicated	At La Jolla, Calif.: Results of cross: Reciprocal	At La Jolla, Calif.: Fertility of F_1: Cross as indicated	At La Jolla, Calif.: Fertility of F_1: Reciprocal
L. perennis (9) ×								
L. indica (9)	0	0			N	N		
L. cretica (8)	0	0			N	N		
L. cretica (8) ×								
L. indica (9)	0	N			N	N		
L. raddeana (9)	0	0			N	N		
L. virosa (9)	0	N			N	N		
L. graminifolia (17)	0	0			N	N		
L. raddeana (9) × *L. indica* (9)	I	0	I I		N	N		
L. canadensis (17) ×								
L. tatarica (9)	I	I	S		N	N		
L. muralis (9)	0	0			N	N		
L. floridana (17)	I	N	I I		0	0		
L. spicata (17)	I	I	I	I	N	N		
L. raddeana (9)	I	N			N	N		
L. graminifolia (17) ×								
L. canadensis (17)	I	I	I	I	I		I	
L. indica (9)	I	N	I I		N	N		
L. laciniata (9)	I	N	S		I	N	S	
L. raddeana (9)	I	N	S		N	N		
L. muralis (9)	I	N	S		N	N		
L. floridana (17)	I	I	I I	I I	0	0		
L. tatarica (9)	I	N	S		N	N		
L. floridana (17) ×								
L. bourgaei (8)	N	N			0	0		
L. indica (9)	I	0	S		N	N		
L. raddeana (9)	I	N	S		N			
L. spicata (17) ×								
L. graminifolia (17)	I	N	S		N	N		
L. indica (9)	I	N	I I		N	N		
L. tatarica (9)	I	N	S		N	N		
L. raddeana (9)	I	N	S		N	N		

Of the 81 cross-pollinations reported in this paper only 29 were successful. Only 10 of the 29 successful crosses produced fertile embryos and 6 of these were partly self-sterile. Sterility of the hybrids in most cases means self-sterility; in only a few cases were the hybrids tested for sterility by backcrossing or outcrossing.

The character of plants obtained from these interspecific crosses varied greatly. In some cases, as in *Lactuca spicata* × *L. graminifolia*, *L. laciniata* × *L. floridana*, *L. graminifolia* × *L. raddeana*, *L. graminifolia* × *L. muralis*, *L. canadensis* × *L. raddeana*, *L. spicata* × *L. tatarica*, *L. floridana* × *L. raddeana*, the hybrids were all weak, abnormal, freakish plants unable to survive beyond the seedling stage. Some of these weak hybrids had very abnormal leaves, in some cases long and narrow, almost grasslike. In many instances the stem apex appeared to be incapable of growth and elongation. Sometimes the growing point became enlarged.

In some cases the hybrids were all normal except for self-sterility. Where it is stated that the hybrids were strong or vigorous normal plants, reference is made to the general appearance of the plants. The hybrids are indicated as normal if they developed with a fair amount of vigor and showed no tendency to be freakish even though they were self-sterile.

In a number of instances the hybrids resulting from a cross varied from very weak abnormal plants to strong normal-appearing ones. Such variation was noted among the hybrids from *Lactuca tatarica* × *L. indica*, *L. floridana* × *L. tatarica*, *L. graminifolia* × *L. floridana*, *L. canadensis* × *L. indica*, *L. canadensis* × *L. tatarica*, and *L. indica* × *L. laciniata*. Even though most of the hybrids from the last-named cross were strong normal plants, producing fertile seed, two abnormal freaks appeared in the F_1 population.

CROSSES BETWEEN NINE-CHROMOSOME SPECIES

Lactuca sativa (9) × *L. serriola* (9) (fig. 1, *A*). - This is one of the three successful crosses made with the cultivated species, *L. sativa*. *L. sativa* and *L. serriola* cross readily, and the resulting hybrid produces fertile seed, regardless of which species is used as the maternal parent.

Crosses between these two species have been reported previously by Durst (*2*), Ernst-Schwarzenbach (*3*), Whitaker and Jagger (*9*), and Thompson (*8*). In the present studies *Lactuca sativa* was crossed with both lobed and unlobed forms of *L. serriola*. The F_1 hybrids between these two species are strong vigorous plants in which the characteristics of *L. serriola* predominate.

Lactuca sativa (9) × *L. altaica* (9) (fig. 1, *B*). Reciprocal crosses were easily obtained between these species. *L. altaica* is a wild type from Egypt. The F_1 plants were vigorous and in many characters resembled the *altaica* parent. The progenies were highly fertile.

Lactuca sativa and *L. altaica* were probably derived from *L. serriola*, since they have the same chromosome number, cross readily, and are similar to *L. serriola* in a great many morphological characters.

Lactuca sativa (9) × *L. virosa* (9) (fig. 1, *C*). This cross was made by pollinating flowers of *L. sativa* var. Imperial D with pollen of *L. virosa*. Six F_1 plants were produced. Repeated attempts to obtain the reciprocal cross resulted in failure. In most respects the F_1 plants were approximately intermediate between Imperial D and *L. virosa*. They were exceedingly vigorous, some reaching a height of over 12 feet. The annual habit of Imperial D was dominant, and there was some pigmentation in the stem and midrib similar to that found in *L. virosa*; however, there was no pigmentation of the leaf blade proper as is characteristic of *L. virosa*. Like Imperial D, the under surface of the midrib of the leaf was free of spines. All F_1 plants were completely sterile. Attempts to backcross the F_1 hybrids to both parental species resulted in failure. Many attempts at Beltsville to cross these two species both ways failed.

Lactuca tatarica (9) × *L. laciniata* (9) (fig. 1, *D*). Reciprocal hybrids were obtained between these two species. Two populations of hybrids were grown. The hybrid seedlings in both cases were very weak, the basal leaves having a tendency to turn yellow and fall off, leaving only a few terminal leaves to carry on growth. As the stems elongated, more of the lower leaves turned yellow and dropped. A few plants were grown to a height of 1 to 2 feet and almost to the flowering stage, but in every case the plants died before producing flowers. Many of the characters of the *L. tatarica* parent predominated.

Under greenhouse conditions at Beltsville, *Lactuca tatarica* was completely self-sterile. Under field conditions *L. tatarica* set seed at both Beltsville and La Jolla.

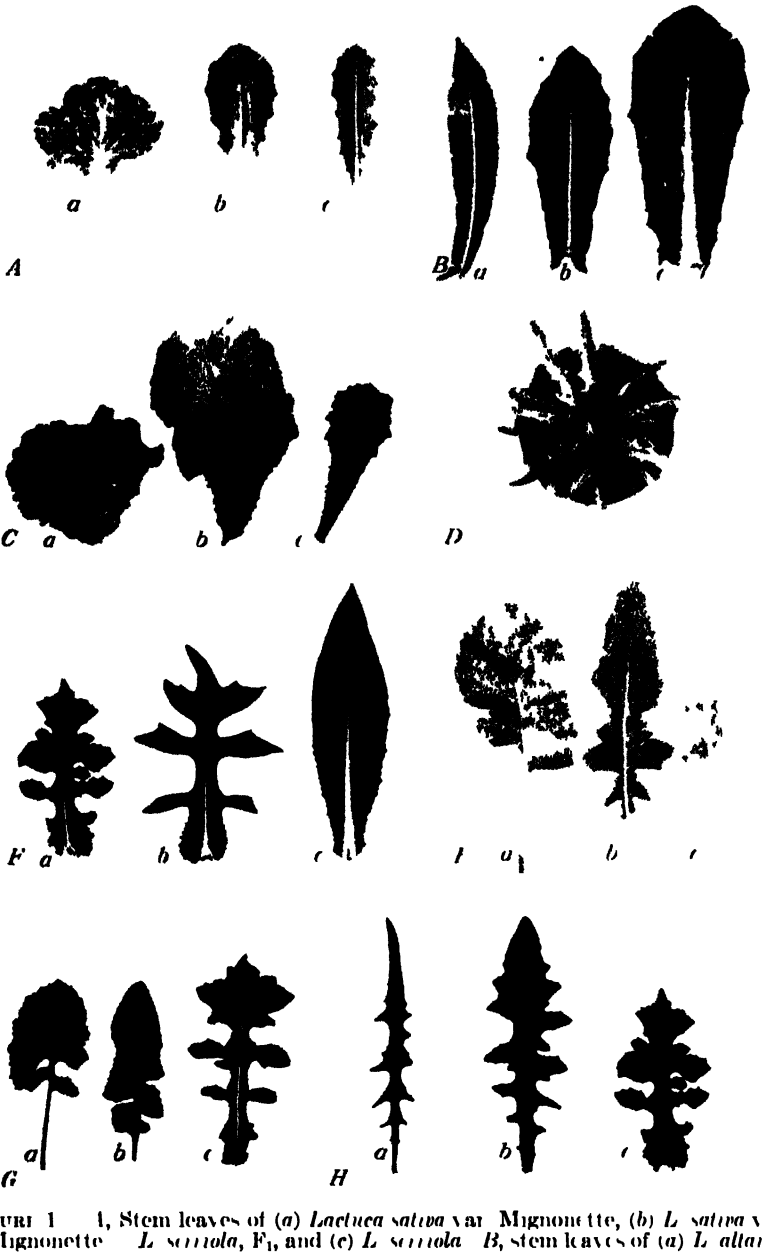

FIGURE 1.—A, Stem leaves of (*a*) *Lactuca sativa* var. Mignonette, (*b*) *L. sativa* var. Mignonette × *L. serriola*, F$_1$, and (*c*) *L. serriola*. *B*, stem leaves of (*a*) *L. altaica*, (*b*) *L. altaica* × *L. sativa* var. Dark Green Cos, F$_1$, and (*c*) *L. sativa* var. Dark Green Cos; *C*, rosette leaves of (*a*) *L. sativa* var. Imperial D, (*b*) *L. sativa* var. Imperial D × *L. virosa*, F$_1$, and (*c*) *L. virosa*. *D*, plant of *L. tatarica* × *L. laciniata*, F$_1$, in the rosette stage; *E*, stem leaves of (*a*) *L. indica*, (*b*) *L. indica* × *L. laciniata*, F$_1$, and (*c*) *L. laciniata*. *F*, rosette leaves of (*a*) *L. raddeana*, (*b*) *L. raddeana* × *L. tatarica*, F$_1$, and (*c*) *L. tatarica*. *G*, rosette leaves of (*a*) *L. raddeana*, (*b*) *L. raddeana* × *L. indica*, F$_1$, and (*c*) *L. indica*. *H*, stem leaves of (*a*) *L. tatarica*, (*b*) *L. tatarica* × *L. indica*, F$_1$, and (*c*) *L. indica*.

Lactuca indica (9) × *L. laciniata* (9) (fig. 1, *E*).—Reciprocal crosses between these species were successful; however, the cross is more easily made by using *L. laciniata* as the maternal parent. The flowers of *L. indica* are easily injured in handling while removing the pollen; hence it is best used as the pollen parent. Two weak, abnormal plants appeared among the hybrids.

The normal F_1 plants from *Lactuca indica* × *L. laciniata* were larger and more vigorous than either parent. The F_1 plants have many of the characters of *L. indica* and look much like this parent. The hybrids were highly fertile and produced abundant seed.

The F_1 hybrid was backcrossed with each of the original parents. Backcrosses with the parents were obtained by pollinating *Lactuca laciniata* with hybrid pollen and by pollinating flowers of the hybrid with pollen from *L. indica*. In both cases the progenies were fertile.

Merrill (4) considers *Lactuca indica* and *L. laciniata* to be synonymous. The results obtained from crossing them indicate that *L. laciniata* should be classed as a variety of *L. indica*.

Lactuca raddeana (9) × *L. tatarica* (9) (fig. 1, *F*). Ten hybrid plants resulted from numerous crosses made between these two species. No hybrids were obtained when *L. tatarica* was used as the maternal parent. The *L. tatarica* plant used in the cross was old and in a very weak condition at the time the crosses were made. This may account for the failure to obtain hybrids when *L. tatarica* was used as the mother plant. Of the 10 hybrids, 8 were weak deformed plants and died while small. The normal hybrids were vigorous and larger than either parent. In the rosette stage they appeared to be intermediate between the parents. The hybrids had a marked tendency to stool and produced numerous stems arising from the crown. The hybrids were all self-sterile.

Lactuca raddeana (9) × *L. indica* (9) (fig. 1, *G*).—From a small population of plants grown from seed obtained by crossing *L. indica* on *L. raddeana*, two hybrids were obtained. Attempts to obtain the reciprocal cross failed. In general appearance the hybrids more nearly resembled *L. indica* than *L. raddeana*, but were less leafy and had longer internodes. The leaves had the dark-green color of *L. indica*. Neither hybrid plant showed any tendency to branch just above the crown as did the *L. raddeana* parent. The heads were intermediate in number of flowers per head. *L. raddeana* is biennial or perennial, and *L. indica* is an annual. The hybrid flowered without overwintering.

The hybrids were at first thought to be completely self-sterile, but a few heads on each plant set seed late as the plants were on the verge of dying. It was found necessary to harvest the seeds a few days before they were mature and to germinate them immediately. If the seeds were permitted to ripen completely on the plant the embryos shriveled and died. No progeny was obtained from mature seed. A few plants are now being grown from seed harvested prematurely.

Lactuca tatarica (9) × *L. indica* (9) (fig. 1, *H*). Seed set very readily in flower heads of *L. tatarica* to which pollen of *L. indica* was applied. The reciprocal cross was also obtained. Several populations of hybrid plants have been grown from crosses between these two nine-chromosome species. A large number of the hybrids have been grown to maturity, but all were completely sterile. In each population of hybrids there were a number of very weak malformed plants. Some of these were grown in pots for several months, but in every case they died without producing a seed stem.

In general appearance the hybrids resembled *Lactuca tatarica* much more than *L. indica*. The foliage had the gray-green color of *L. tatarica*, although the flower heads were a little smaller and the corollas a lighter blue.

CROSSES BETWEEN 17-CHROMOSOME SPECIES

Lactuca canadensis (17) × *L. floridana* (17) (fig. 2, *A*).- Flowers of *L. canadensis* were pollinated with pollen from *L. floridana*. From the seed obtained 15 plants were grown, 3 of which were weak and very deformed. Although kept in pots for several months, they failed to develop. The remaining seedlings were strong and vigorous.

Although both the species used are normally biennial, two of the plants developed seed stems the first season and both proved to be hybrids. Both plants were strong and vigorous, but they were no taller and no heavier stemmed than *L. canadensis* and indicated little hybrid vigor. In general appearance they were intermediate between the two parents. The flowers were intermediate in size, and the corollas were blue of a little lighter shade than those of *L. floridana*.

Both hybrid plants were partially self-sterile, although both produced a few seeds. An F_2 population is being grown.

Lactuca graminifolia (17) × *L. canadensis* (17) (fig. 2, *B* and *C*).- This cross can be readily made in both directions. The F_1 plants are intermediate between the two species in most respects, including height, size and shape of leaf, color and shape of ligules, and color and size of achenes. The writers' observations indicate that the annual habit and the gray color of the pollen grains of *L. graminifolia* are dominant over the biennial habit and the orange-colored pollen of *L. canadensis*. The F_1 was as fertile as either parent.

Lactuca canadensis (17) × *L. spicata* (17) (fig. 2, *D*).- From a population of 25 plants grown from seed resulting from this cross, with *L. spicata* as the pollen parent, 20 hybrids were obtained. In the rosette stage the hybrids resembled *L. spicata* more than *L. canadensis*. The fertility of the hybrids is not known, since none have reached the flowering stage. The reciprocal cross was easily obtained.

Lactuca morssii Robinson, as described in Gray's Manual (*7*), is generally considered to be a hybrid between *L. spicata* and *L. canadensis*. This form is found at low elevations in parts of Maine and Massachusetts. Wiegand and Eames (*10*) report natural hybrids between these two species occurring in the wild in New York State.

Lactuca spicata (17) × *L. graminifolia* (17). Eighteen hybrid plants were obtained from this cross, *L. graminifolia* being used as the pollen parent. All of the hybrids were abnormal, weak plants unable to survive beyond the seedling stage. The reciprocal cross failed.

Lactuca graminifolia (17) × *L. floridana* (17) (fig. 2, *E*). Reciprocal crosses were obtained between these two species. All of the 17 hybrids produced by using *L. graminifolia* pollen were weak, abnormal plants. Of the 22 hybrids obtained by using *L. floridana* as the pollen parent, 11 were abnormal and freakish and 11 were normal. The plants that flowered were partially fertile.

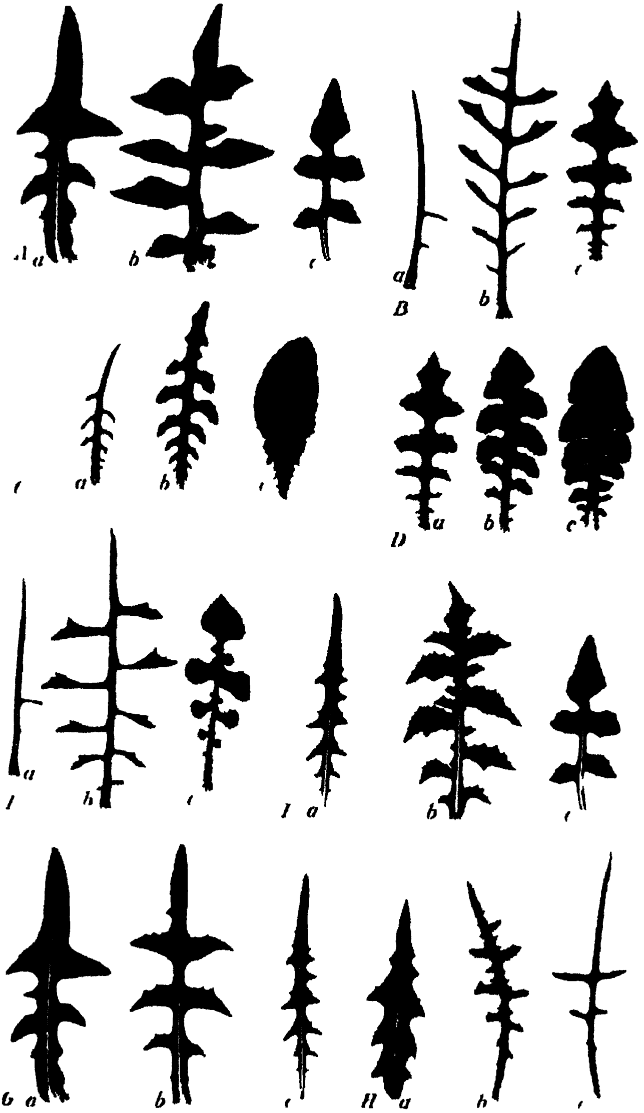

FIGURE 2.—A, Stem leaves of (*a*) *Lactuca canadensis* var. *latifolia*, (*b*) *L. canadensis* var. *latifolia* × *L. floridana*, F_1, and (*c*) *L. floridana*. *B*, stem leaves of (*a*) *L. graminifolia*, (*b*) *L. graminifolia* × *L. canadensis* var. *latifolia*, F_1, and (*c*) *L. canadensis* var. *latifolia*. *C*, rosette leaves of (*a*) *L. graminifolia*, (*b*) *L. graminifolia* × *L. canadensis* var. *integrifolia*, F_1, and (*c*) *L. canadensis* var. *integrifolia*. *D*, rosette leaves of (*a*) *L. canadensis* var. *latifolia*, (*b*) *L. canadensis* var. *latifolia* × *L. spicata*, F_1, and (*c*) *L. spicata*. *E*, rosette leaves of (*a*) *L. graminifolia*, (*b*) *L. graminifolia* × *L. floridana*, F_1, and (*c*) *L. floridana*. *F*, stem leaves of (*a*) *L. tatarica*, (*b*) *L. tatarica* × *L. floridana*, F_1, and (*c*) *L. floridana*. *G*, stem leaves of (*a*) *L. canadensis* var. *latifolia*, (*b*) *L. canadensis* var. *latifolia* × *L. tatarica*, F_1, and (*c*) *L. tatarica*. *H*, stem leaves of (*a*) *L. graminifolia*, (*b*) *L. graminifolia* × *L. tatarica*, F_1, and (*c*) *L. tatarica*.

CROSSES BETWEEN 9- AND 17-CHROMOSOME SPECIES

Lactuca tatarica (9) × *L. floridana* (17) (fig. 2, *F*).—*L. tatarica*, an annual species having 9 pairs of chromosomes, was used as the maternal parent in a cross with the biennial species *L. floridana*, having 17 pairs of chromosomes. Both of these species are blue-flowered. The flowers of *L. tatarica* are much larger than those of *L. floridana* and the anther sheath is less pigmented. Both have pinnately lobed leaves.

Eight hybrid plants were grown from the seed obtained from the cross-pollinations. All were self-sterile and behaved as annuals. As in most of the cases of self-sterility observed in *Lactuca*, these plants continued to grow and produced flowers over a long period.

The hybrid plants showed marked hybrid vigor in the rosette stage but did not develop extremely large seed stems. The flowers were blue and about the same size as those of *Lactuca tatarica*. The dark pigmentation of the anther sheaths in *L. floridana* was dominant. The leaves were a gray green similar to those of *L. tatarica*, but they were much larger and broader than in either parent. The annual habit of *L. tatarica* was dominant.

Cytological examination of root-tip material showed the somatic cells of the hybrids to have 26 pairs of chromosomes.

Lactuca canadensis (17) × *L. tatarica* (9) (fig. 2, *G*). Several flower heads of the 17-chromosome species *Lactuca canadensis* were cross-pollinated with the 9-chromosome species *L. tatarica*. Of the plants grown from these cross-pollinations, 34 proved to be interspecific hybrids having 26 pairs of chromosomes. All hybrids were completely self-sterile. The plants were quite uniform in general appearance and resembled the *L. tatarica* parent in many characteristics. The parent plants apparently were somewhat heterozygous. Although the hybrids were uniform in general appearance, close examination indicated considerable variation. Of the 34 hybrids, 5 were small and weak as compared with the average of the population, and 3 of these died before reaching the flowering stage. All the hybrids carried anthocyanin pigment in their leaves but were not identical in type of pigmentation; 18 were of the tinged type [5] and 16 were more heavily pigmented, approaching the red type. The green of the leaves approached the gray green of the *L. tatarica* parent. Some of the hybrids developed a thick brown epidermis that had a tendency to crack as the plants reached maturity. Counts on the 16 mature plants showed 10 to have this character in some degree. All the plants having this thick outer layer appeared deformed.

The flowers were intermediate in size. The color of the corolla was nearly the same shade of blue as that of *Lactuca tatarica*, showing dominance of blue over the yellow color characteristic of *L. canadensis*. These plants produced an enormous number of flowers over a long period, but no seed developed.

Sprouting from the roots, a characteristic of *Lactuca tatarica*, appears to be dominant. The seed stems were removed from 16 plants after they had been in flower for several weeks. Most of them had developed sprouts from the roots 2 months after the seed stem was removed. The hybrids did not show a tendency to sprout or stool from the crown as *L. tatarica* does. The annual habit was dominant. Reciprocal hybrids were obtained, but none were grown to maturity.

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[5] The leaf color types were based on the classification by Thompson *(8)*.

Lactuca graminifolia (17) × *L. tatarica* (9) (fig. 2, *H*).—The third cross between a 17-chromosome species and the 9-chromosome species *Lactuca tatarica* was obtained by applying pollen from *L. tatarica* to flowers of *L. graminifolia*. Fifteen hybrid plants were grown from this cross, all of which were sterile. As in the crosses of *L. tatarica* with *L. canadensis* and *L. floridana*, the hybrids more nearly resembled the *L. tatarica* parent. The gray green of *L. tatarica* also appeared to be dominant in this case. The plants were a little larger and more vigorous than either parent.

The flowers were intermediate in size and of about the same shade of blue as those of the *Lactuca tatarica* parent. Some of the hybrids sprouted or stooled at the crown like *L. tatarica*, while others produced only a single stem characteristic of *L. graminifolia*. Like both parent species, the hybrids behaved as annuals, producing flowers the first season. However, there was a great difference in time of seed-stem elongation among the hybrids, nine of them producing seed stems much earlier than the remaining six. The individual plants within the two groups elongated at about the same time and rate. Plants that stooled were slower to develop seed stems than single-stem plants.

Lactuca laciniata (9) × *L. floridana* (17). Eighty-three hybrid plants were grown from seed obtained by applying pollen of *Lactuca floridana* to flowers of *L. laciniata*, and 11 were obtained from the reciprocal cross. The hybrids were all very weak and unable to develop beyond the seedling stage, regardless of which way the cross was made The hybrid seedlings developed five to six leaves, then turned yellow and died. These plants were readily distinguished from either parent, and there was no doubt of their being hybrids. Progenies from this cross were planted at three different dates, but in each case the hybrids were weak and died after developing a few leaves.

Lactuca floridana (17) × *L. indica* (9) (fig. 3, *A*). Several flower heads of *Lactuca floridana* were crossed with *L. indica*. Only one hybrid plant was obtained from the seed from this cross. The plant was intermediate in size and resembled the *L. indica* parent more than *L. floridana*. It had anthocyanin spots on the leaves like *L. indica*, but they were much larger and of a more intense red. The flowers were intermediate in size and the corollas blue, of about the shade of *L floridana*. The reciprocal cross failed.

Lactuca floridana generally behaves as a biennial, and *L. indica* is annual in habit. The hybrid plant was an annual and completely sterile.

Lactuca graminifolia (17) × *L. raddeana* (9). Fifty-three plants were grown from seed resulting from the application of pollen of *Lactuca raddeana* to flowers of *L. graminifolia*. Of these, 48 were hybrids. The hybrid plants were all very weak and died in the seedling stage.

Lactuca graminifolia (17) × *L. muralis* (9). Pollen of *L. muralis* applied to flowers of *L. graminifolia* resulted in 9 hybrids out of a population of 14 plants. All the hybrids were weak and unable to survive beyond the seedling stage. This is probably the widest cross obtained so far, for the two species differ greatly in many morphological characters.

Lactuca canadensis (17) × *L. raddeana* (9). A population of 23 plants was grown from seed obtained by applying pollen of *L. raddeana*

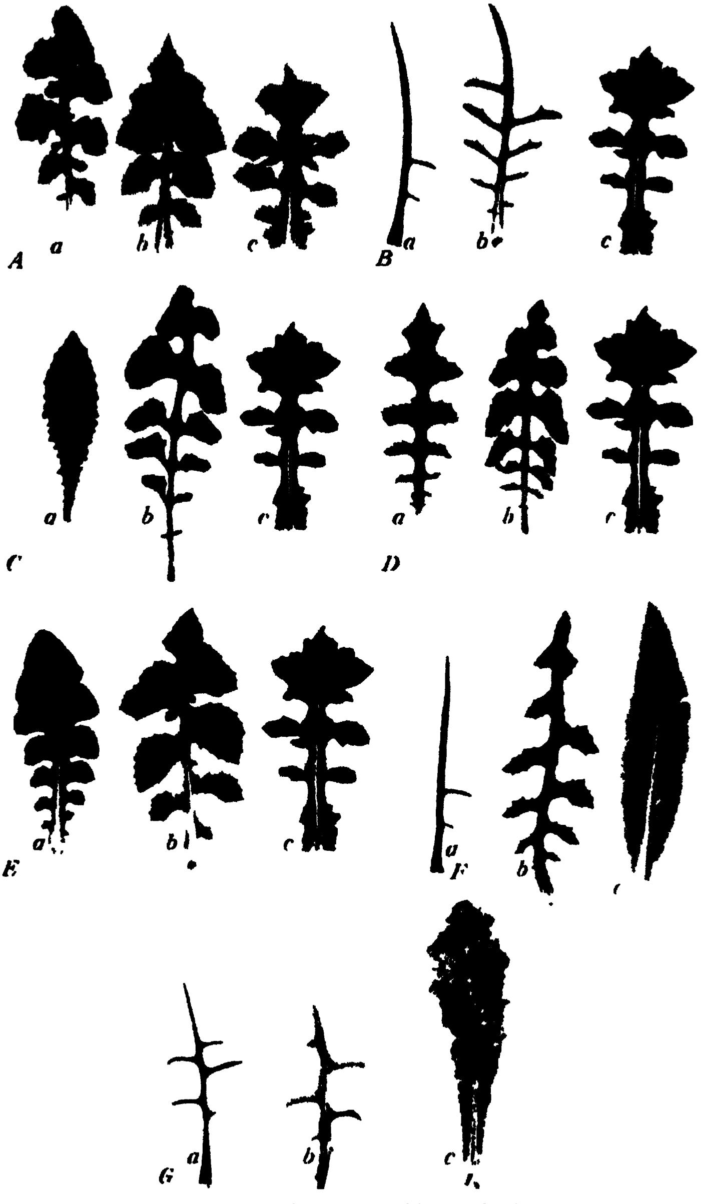

FIGURE 3. *A*, Stem leaves of (*a*) *Lactuca floridana*, (*b*) *L. floridana* × *L. indica*, F_1, and (*c*) *L. indica*; *B*, stem leaves of (*a*) *L. graminifolia*, (*b*) *L. graminifolia* × *L. indica*, F_1, and (*c*) *L. indica*; *C*, rosette leaves of (*a*) *L. canadensis* var. *integrifolia*, (*b*) *L. canadensis* var. *integrifolia* × *L. indica*, F_1, and (*c*) *L. indica*; *D*, rosette leaves of (*a*) *L. canadensis* var. *latifolia*, (*b*) *L. canadensis* var. *latifolia* × *L. indica*, F_1, and (*c*) *L. indica*; *E*, rosette leaves of (*a*) *L. spicata*, (*b*) *L. spicata* × *L. indica*, F_1, and (*c*) *L. indica*; *F*, rosette leaves of (*a*) *L. graminifolia*, (*b*) *L. graminifolia* × *L. laciniata*, F_1, and (*c*) *L. laciniata*; *G*, rosette leaves of (*a*) *L. graminifolia*, (*b*) *L. graminifolia* × *L. virosa*, F_1, and (*c*) *L. virosa*.

to flower heads of *L. canadensis*. Of the 23 plants 10 were very weak hybrids and all of these died in the seedling stage. Normal hybrids were not obtained.

Lactuca graminifolia (17) × *L. indica* (9) (fig. 3, *B*). From seed obtained by applying pollen of *L. indica* to flowers of *L. graminifolia*, 29 hybrids were obtained. All were normal, vigorous plants, producing small, dark reddish-blue flowers similar to those of *L. graminifolia*. Some of these hybrids produced flowers and a few seeds, indicating partial fertility.

Lactuca spicata (17) × *L. tatarica* (9). Four hybrids obtained from this cross with *L. tatarica* as the pollen parent were all weak, abnormal plants and were unable to survive beyond the seedling stage.

Lactuca canadensis (17) × *L. indica* (9) (fig. 3, *C* and *D*). All but 5 of 19 hybrids from this cross with *L. indica* as the pollen parent were weak, abnormal plants and died in the seedling stage. Some of the normal hybrid plants have come to the flowering stage and are self-sterile.

Lactuca spicata (17) × *L. raddeana* (9). Five hybrid plants were obtained from seed resulting from the application of *L. raddeana* pollen to flowers of *L. spicata*. All were weak and died in the seedling stage.

Lactuca spicata (17) × *L. indica* (9) (fig. 3, *E*). Fifteen hybrid plants were obtained from a cross between *L. spicata* and *L. indica* in which the latter was used as the pollen parent. The hybrids were strong, vigorous plants. In the seedling stage they looked more like *L. indica* than *L. spicata*. The leaves were more lobed than those of *L. spicata* and had large spots of dark-red anthocyanin. Some of the hybrids have reached the flowering stage and one has produced a few seeds.

Lactuca floridana (17) × *L. raddeana* (9). Sixteen hybrid plants were obtained from this cross, *L. raddeana* being used as the pollen parent. All the hybrids were weak, abnormal plants and died in the seedling stage.

Lactuca graminifolia (17) × *L. laciniata* (9) (fig. 3, *F*). Seven hybrid plants were obtained from this mating at La Jolla, Calif. All of them had considerable dark-red pigment along the midrib of the leaf, similar to that on *L. laciniata*. These plants were unmistakable hybrids and were very weak. They were susceptible to a variety of foliage diseases, and none developed past the rosette stage. Twenty-two hybrids were obtained at Beltsville, Md., all but one of which were weak and died in the seedling stage.

Lactuca graminifolia (17) × *L. virosa* (9) (fig. 3, *G*). One F_1 plant was obtained by pollinating flowers of *Lactuca graminifolia* with *L. virosa* pollen. This plant was similar to *L. graminifolia* in most respects. It was an annual and of about the same height and shape as *L. graminifolia*. It was positively identified as a hybrid by the fact that the leaf blades were characterized by numerous small patches of pigment on the upper surface of the leaf blade, like *L. virosa*. The flowers had the purple-colored ligules of *L. graminifolia*, but the purple was much lighter in the hybrid.

DISCUSSION

The results obtained from the interspecific crosses reported above give some interesting information regarding the genetic relationship of certain species within the genus *Lactuca.*

The breeding behavior of the 18 forms studied indicates that, excepting the 8-chromosome species *Lactuca bourgaei* and *L. cretica* and the 9-chromosome species *L. muralis, L. perennis,* and *L. chondrillaeflora,* all belong to three quite distinct compatibility groups: (1) The group hereinafter called the *serriola* group, consisting of *L. serriola, L. sativa, L. saligna, L. altaica,* and *L. virosa*; (2) the group hereinafter called the *indica* group, consisting of *L. indica, L. laciniata, L. raddeana,* and *L. tatarica*; and (3) the group consisting of the four 17-chromosome species *L. canadensis, L. spicata, L. floridana,* and *L. graminifolia.*

In group 1, *Lactuca sativa, L. serriola,* and *L. altaica* are indicated to be varieties of the same species, since they cross readily in either direction, with fertile progenies, and have many morphological characters in common. In group 2, *L. laciniata* is indicated to be a variety of *L. indica,* since the two forms have many morphological similarities and cross readily, giving fertile progenies.

Though hundreds of cross-pollinations were made between species in group 1 and those in group 2, not a single hybrid was obtained, suggesting a remote genetic relationship between these two groups of 9-chromosome species.

Although *Lactuca indica* and *L. laciniata* are morphologically closely related to *L. serriola, L. saligna,* and *L. virosa,* and are taxonomically placed in the subgenus *Serriola,* there is evidence of some genetic relationship with *L. raddeana* and *L. tatarica* in the subgenus *Mulgedium.*

The four species with 17 chromosomes have been separated by taxonomists into the two subgenera *Serriola* and *Mulgedium* largely on the characters of the achenes and involucres. *Lactuca canadensis* and *L. graminifolia* have been assigned to the subgenus *Serriola* and *L. floridana* and *L. spicata* to the subgenus *Mulgedium.* Four crosses were obtained between these two subgenera, namely, *L. canadensis* × *L. floridana, L. canadensis* × *L. spicata, L. graminifolia* × *L. floridana,* and *L. spicata* × *L. graminifolia.* The first-named cross gave some fertile progenies. Fertile hybrids of the second cross, *L. canadensis* × *L. spicata,* have very likely developed in nature (*7, 10*). The third cross, *L. graminifolia* × *L. floridana,* gave partially fertile progenies. The fourth cross, *L. spicata* × *L. graminifolia,* is known to produce self-sterile progenies. A fifth cross between two 17-chromosome species in the subgenus *Serriola* (*L. graminifolia* × *L. canadensis*) gave fertile progenies. These results indicate a close genetic relationship among all four of these species.

Sixteen successful crosses were made between 17- and 9-chromosome species. All of these except one involved 9-chromosome species within the *indica* group. The only hybrid plant obtained between a 17-chromosome species and the *serriola* group was at La Jolla, Calif., between *Lactuca graminifolia* and *L. virosa,* when *virosa* pollen was used. Table 1 shows the many attempted crosses between the 17-chromosome group and the 9-chromosome *serriola* group. In most cases at least 10 flower heads were cross-pollinated, and in many cases a large number of crosses were tried. Most of these attempts were made under conditions favorable for fertilization, yet only the one hybrid plant was obtained.

The ease with which crosses were obtained between the 17-chromosome species and the 9-chromosome *indica* group, as compared with the almost total failure to obtain crosses between the 17-chromosome group and the *serriola* group, indicates a much closer genetic relationship between the 17-chromosome group and the *indica* group than between the 17-chromosome group and the *serriola* group. This is closely paralleled by the similarity between the two groups in gross morphology.

It is generally assumed that the 17-chromosome species, *Lactuca canadensis*, *L. spicata*, *L. floridana*, and *L. graminifolia*, are amphidiploids, having arisen from hybrids between 8- and 9-chromosome species. If this assumption is correct, the behavior of the interspecific hybrids herein reported indicates that the 9-chromosome species contributing to the amphidiploids were more likely to have been members of the *indica* group or closely related species than of the *serriola* group.

The sterility of many of the interspecific hybrids prevents any extensive study of the inheritance of characters; however, an apparent dominance of certain characters was noted in the F_1 populations. The annual is dominant over the biennial habit, as indicated by the matings of *Lactuca canadensis* × *L. tatarica*, *L. floridana* × *L. tatarica*, and *L. floridana* × *L. indica*. *L. canadensis* and *L. floridana* are both biennials—at least the stocks used in these studies were biennials; *L. indica* and *L. tatarica* are annuals. All of the hybrids were annuals.

Where lobed-leaved species were crossed with lanceolate nonlobed species, the hybrids were always lobed, indicating dominance of this character.

Light-colored, gray, or whitish color of pollen was dominant over orange in *Lactuca graminifolia* × *L. canadensis*, *L. canadensis* × *L. tatarica*, and *L. graminifolia* × *L. indica* crosses, in which *L. graminifolia* and *L. tatarica* have light-colored pollen.

The gray-green foliage color of *Lactuca laciniata* was dominant over other foliage greens in *L. laciniata* × *L. indica* and *L. floridana* × *L. laciniata*.

The tendency to sprout from the roots, characteristic of *Lactuca tatarica*, appeared to be a dominant character in the hybrids *L. canadensis* × *L. tatarica*, *L. floridana* × *L. tatarica*, *L. tatarica* × *L. indica*, and *L. graminifolia* × *L. tatarica*.

The susceptibility of *Lactuca laciniata* to certain foliage diseases appeared to be dominant, as most of the hybrids in which this species was a parent were subject to similar maladies, which added to the difficulties of growing the hybrids to the flowering stage.

Anthocyanin pigment in the foliage, stems, and flowers was in every case dominant over its absence.

The membranous margins on the achenes of *Lactuca canadensis* were dominant in *L. canadensis* × *L. tatarica* and *L. canadensis* × *L. floridana* crosses. Although *L. canadensis* × *L. tatarica* hybrids were sterile, examination of the infertile ovaries showed them to have this type of margin.

Few weak spines, or no spines, on the under side of the midrib of the leaf seem to be dominant over many coarse spines.

In most crosses between 9- and 17-chromosome species, hybrids were obtained only when the 9-chromosome species was used as the pollen parent. In fact, it is generally considered difficult to effect crosses between species of different chromosome number when the species having the larger number is used as the pollen parent. Müntzing (*5*) discusses at some length this question of crosses between species being more successful when the one having the larger chromosome number is used as the maternal parent, and he offers double fertilization as the possible explanation. In two cases herein reported two species of different chromosome number were crossed in both directions, with hybrids resulting. These were the *Lactuca floridana* × *L. laciniata* and *L. canadensis* × *L. tatarica* crosses.

It is not assumed that these investigations reveal all the possible relationships that exist among the species studied. No doubt, under more nearly optimum conditions for certain of these species, crosses might be effected that are herein reported to have failed. Fertile embryos under certain conditions might develop in hybrids here reported to be completely self-sterile. The hybrid *Lactuca raddeana* × *L. indica* is an example; if the two plants had not been observed carefully in the very last stages of maturity and the immature embryos tested for viability, these hybrids would have been reported as completely self-sterile. Hundreds of crosses were made between *L. virosa* and other members of this nine-chromosome group before hybrids were obtained with *L. serriola*.

The extent of these investigations and the results obtained would seem to establish the close relationship among the species within the two nine-chromosome groups and the more remote relationship, if not complete incompatibility, of the two groups.

These studies have contributed some tangible support to the assumed amphidiploid nature of the 17-chromosome species and give substantial evidence that the 9-chromosome species involved in the synthesis of the amphidiploids was a member or were members of the *indica* group or closely related species. Similarly, the 9-chromosome species included in the *serriola* group are practically eliminated as possible contributors to the synthesis of the amphidiploids.

The results of these studies of interspecific hybridization have one discouraging aspect from the viewpoint of the lettuce breeder; that is, the apparent genetic incompatibility of the cultivated form *Lactuca sativa* with many of the other species of the genus. Hybridization of *L. sativa* appears to be possible only with *L. serriola* and possibly with some very closely related forms. Some of the most promising material for breeding for resistance to certain diseases is in species too remotely related genetically to *L. sativa* to permit hybridization that will result in fertile progenies.

SUMMARY

Results from 81 attempted interspecific crosses among 18 species of the genus *Lactuca* are presented. These crosses involved two 8-chromosome, twelve 9-chromosome, and four 17-chromosome species.

Of the 81 attempted crosses, 29 were successful in producing viable embryos and hybrid seedlings.

Eleven of the successful crosses were self-fertile or partly so. The remaining 18 produced either weak, abnormal seedlings unable to

survive beyond the infantile or rosette stage, or strong, vigorous plants that developed more or less normally but were completely self-sterile.

The four 9-chromosome species *Lactuca indica*, *L. laciniata*, *L. tatarica*, and *L. raddeana* were found to be closely related genetically and capable of crossing among themselves. Except in the case of *L. indica* × *L. laciniata* and *L. raddeana* × *L. indica*, the hybrids were self-sterile.

The nine-chromosome species *L. sativa*, *L. serriola*, *L. altaica*, *L. saligna*, and *L. virosa* were found by breeding behavior to be closely related and capable of crossing among themselves. Some of the hybrids resulting from cross-pollination within this group were as fertile as either parent; others were completely self-sterile.

Although many crosses were attempted between species from the two groups of nine-chromosome species listed above, no hybrids were obtained.

The four 17-chromosome species studied were found to be closely related genetically and capable of crossing among themselves. Some of these crosses resulted in fertile or partially fertile hybrids.

Sixteen attempted crosses between 17- and 9-chromosome species were successful in producing fertilized embryos. All but 2 of these 16 crosses involved a 9-chromosome species in the *indica* group. Crosses between the 17-chromosome species and the 9-chromosome species of the *serriola* group failed in every case but 1, that of *Lactuca graminifolia* × *L. virosa*.

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SULFUR AND TRACE-ELEMENT NUTRITION OF ASPERGILLUS NIGER[1]

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INTRODUCTION

Though it is known that sulfate sulfur disappears in the green plant to reappear as disulfide (cystine) and sulfhydryl (cysteine), little is known concerning this process of reduction, or of the intermediate compounds involved. Nightingale et al. (*12*)[3] found that sulfate is reduced to sulfite and apparently to sulfhydryl in the comparatively alkaline phloem region of roots and tops. Neither Heisrich (*5*) nor Mothes (*10*) was able to bring about reduction of sulfate with macerated green tissue. However, reduction of sulfate to disulfide was reported by Hammett and Reynolds (*4*) to take place with extracts of *Phaseolus vulgaris* root tips. They considered the process to be enzymatic, since the heated extracts did not cause a diminution in sulfate.

According to summaries by Pfeffer (*13*), Jost (*6*), and Miller (*9*), green plants require sulfate and are unable to assimilate any of its reduction products. Recently, however, increased yields have been found to occur with atmospheric sulfur dioxide (0.1 to 0.2 p.p.m.) by Setterstrom (*14*). Though the possibility exists that oxidation of sulfur dioxide occurred prior to use, the experimental data would indicate the desirability of a restudy of sulfur utilization in green plants. There can be little doubt of the presence in plants of sulfur compounds intermediate in oxidation between sulfhydryl and sulfate (*10*, *12*). It appears improbable that sulfur is introduced into organic compounds as sulfate by the green plant without previous reduction.

The situation is quite different with respect to fungi. These have been grown successfully with a large number of sulfur compounds (*2*, *7*, *18*, *19*). Here, again, insufficient evidence is available to determine the course of sulfur assimilation except in a general way. Mothes (*10*) was able to determine, however, that the sulfur metabolism of *Aspergillus niger* parallels that of green plants, whereas it diverges from that of the yeasts. Fuller information, in his opinion, may reveal that specific differences in sulfur metabolism also exist between green plants and fungi.

A general survey of the relation between constitution and assimilability of sulfur compounds was therefore undertaken with this fungus.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 126.

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A fuller knowledge of sulfur metabolism in *Aspergillus niger* should prove of value in working out that of the green plant. The absence of rapid and sensitive methods for the chemical identification of suspected intermediary forms of sulfur made necessary the adoption of comparisons upon an exactly quantitative basis. This was accomplished by measuring assimilability of sulfur by the yield obtained with 25 mg. of sulfur per liter at 35° C., after 4 days of growth in an optimum solution. The maximum dry weight (1,100 to 1,300 mg.) obtained with sulfate sulfur served as a standard of comparison.

The sulfur compounds studied fall into five groups of which the known reduction products of sulfuric acid are the most important. Other sources of inorganic sulfur are the thionic acids. The naturally occurring sulfur compounds, such as cystine and methionine and their derivatives, fall into a special category. Lastly, there are the miscellaneous organic sulfur compounds, some of natural occurrence, including the oxidation-reduction series of mercaptan sulfonate and of sulfide-sulfone. These are treated separately. These substances are, on the whole, the simple sulfur-containing compounds into which the more complex forms would be transformed before assimilation under ordinary conditions.

The general assumption underlying the experimental procedures and interpretation of data is that the initial stages of sulfur assimilation are essentially a process of chemical reduction (digestion). Armstrong (*2*) and others have found *Aspergillus niger* to be capable of carrying this reduction to the production of elemental sulfur. Inability of the fungus to grow when supplied with sulfur in a reduced form was assumed to imply, in addition, that oxidation of nutritive sulfur is a matter of difficulty for the fungus.

Paralleling the studies on sulfur assimilation were others with the trace elements iron, zinc, copper, manganese, molybdenum, and gallium. The effects of a deficiency of each of these elements were studied with a variety of sulfur compounds in an attempt to determine whether a specific role was played by one of these elements in the (enzymatic) reduction of sulfur compounds. A relationship of this sort has been found between nitrate reduction and molybdenum (*17*).

MATERIAL AND METHODS

Aspergillus niger Van Tiegh. (No. 215 1247 in the collection of Dr. Charles Thom) was grown on 50-cc. portions of a nutrient solution in 200-cc. Erlenmeyer flasks at 35° C. for 4 days. The nutrient solution consisted of redistilled water 1,000 cc., sucrose 50 gm., reagent ammonium nitrate 2.06 gm., reagent dipotassium phosphate 0.35 gm., and reagent magnesium sulfate ($7H_2O$) 0.25 gm. This solution contained 25 mg. of sulfur per liter, or 1.25 mg. of sulfur per culture. Practically spectroscopically pure iron, zinc, copper, manganese, molybdenum, and gallium were supplied as chlorides in concentrations of 0.30, 0.30, 0.075, 0.075, 0.02, and 0.02 mg. per liter, respectively. The sucrose contained 0.00087 percent of ash. All cultural vessels and accessory apparatus were of transparent quartz, except only the boiling flask of the still and the storage bottle for redistilled water, which were of silica ware. Inoculation was by means of a spore suspension.

When nutrient-solution purification (*15*) was employed, the ammonium nitrate was increased to 2.60 gm. per liter, the dipotassium phosphate to 0.70 gm., and the magnesium sulfate to 1.10 gm. in the above formula. After the addition of 1 gm. of calcium carbonate and heating to 100° C. for 20 minutes (steamer), the solution was filtered through a fritted quartz crucible of No. 4 porosity. Trace elements were added subsequently.

In solutions provided with other than sulfate-sulfur, magnesium was supplied as $MgCl_2.6H_2O$, 0.21 gm. per liter, or 0.91 gm. per liter when the solution was purified with calcium carbonate. Sulfur compounds other than sulfate were also supplied in concentrations sufficient to provide 25 mg. of sulfur per liter, or, if subjected to nutrient-solution purification, 50 mg. of sulfur per liter

In performing the starch tests a drop of N/20 iodine solution was placed on the reverse of the mycelial felts during filtration, washed several times with water, and examined for starch at × 10 magnification after the lapse of an hour or more. A quinhydrone electrode was used to determine acidity. Yields are given per culture of 50 cc., the mycelial felts having been dried at 103° C. overnight after filtration with fritted glass crucibles of No. 3 porosity.

The sulfur compounds used were reagent chemicals or the purest available.

ASSIMILABILITY OF INORGANIC SULFUR

The maximum yields obtained with sulfate and its products of reduction are tabulated in table 1. It can be seen that as sulfate was successively reduced to sulfite, hyposulfite, sulfoxylate, and sulfide, no diminution in assimilation occurred until the sulfide was formed. Sulfoxylate, though unobtainable, is included here because sodium hyposulfite hydrolyzes in slightly acid solution to form an equimolecular mixture of sulfoxylic and sulfurous acids, which is assimilated as readily as sulfurous acid alone. Utilization of sodium hyposulfite is therefore considered to depend wholly on the formation of sulfoxylate. The bisulfite residue is presumably reduced again to sodium hyposulfite by the fungus. It does not seem probable from the chemical data as summarized by Mellor (8) that sulfoxylate has been produced directly by reduction of hyposulfite, though sulfide can be so formed. It is clear, at any rate, that sodium sulfoxylate should also be capable of giving maximum yields, and that it is the lowest state of oxidation in which inorganic sulfur can be utilized efficiently.

TABLE 1 *Growth data for Aspergillus niger after 4 days at 35° C. with inorganic compounds providing 25 mg of sulfur per liter of solution*

Source of sulfur	Formula	Yield per 2.5 gm sucrose	Sporulation[1]
		Mg	
Potassium persulfate	$K_2S_2O_8$	716.2	2
Sodium sulfite	Na_2SO_3	1,173.0	8
Sodium bisulfite	$NaHSO_3$	1,099.3	8
Sodium hyposulfite	$Na_2S_2O_4.2H_2O$	1,101.3	9
Sodium sulfoxylate	Na_2SO_2	( )	
(Hydrogen sulfide)	H_2S	(912.5)	6
Sodium disulfide (pH=6.62)	Na_2S_2	602.3	8
Ferrous sulfide	FeS	166.1	9
Sodium sulfide (pH=6.70)	$Na_2S.9H_2O$	256.4	10
Sodium thiosulfate	$Na_2S_2O_3.5H_2O$	1,029.8	4
Sodium dithionate	$Na_2S_2O_6$	2.1	0
Sulfamic acid	NH_2SO_3H	949.3+	4

[1] Sporulation is rated as 0 (sterile) to 10 (maximum). All spores were black.
[2] Maximum.

Results with sulfide and disulfide require further explanation (see also tables 2 and 5). An old sample of sodium sulfide gave maximum yields, as did also the sodium disulfide formed by solution of the calculated amount of sulfur in a 9-percent solution of the sulfide. Inasmuch as this same sample has been used in a previous study (*16*) and found unserviceable as a source of sulfur, additional tests were made to clear up the discrepancy. The data obtained indicated that the sulfur of old and presumably altered (oxidized) samples of sodium sulfide (or disulfide prepared with it) was fully assimilable. Fresh samples gave poor growth, showing symptoms of sulfur deficiency. The transformation of nonassimilable sulfur of sodium sulfide and disulfide to assimilable sulfur was found to be accelerated even by slight alkalinity. Yields with disulfide were uniformly greater than with sulfide. This difference will be discussed in connection with tables 2 and 5.

Growth with sulfur as hydrogen sulfide was almost optimum, but little weight is placed on these data, inasmuch as passage of gas through the nutrient solution (5 minutes) caused precipitation of much sulfur. As will be noticed, ferrous sulfide served poorly as a source of sulfur, though decomposed by the organism, unless a large excess was present. Turbidity of the substrate in the latter case would indicate that a similar decomposition of hydrogen sulfide presumably occurred as with passage of the gas.

An oxidation product of sulfuric acid, namely, persulfate, proved freely available as a source of sulfur, and it is believed that it would have given maximum yields with adjustment of other components of the nutrient solution. A like interpretation of the results with sulfamic acid is also probable. Only two of the six known thionic acids could be tried as sources of sulfur, namely, thiosulfate and dithionate. Sodium thiosulfate gave maximum yields when used as a source of sulfur. Practically no growth occurred with sodium dithionate.

TABLE 2.—*Effect of acidity and age of sample on the utilization of sulfide and disulfide by Aspergillus niger (4 days' growth at 35° C.)*

Source of sulfur	Initial acidity of nutrient solution	Yield per 25 gm. of sucrose	Starch¹ in mycelium	Sporulation²
	pH	*Mg.*		
Sodium sulfide (old sample)	6.62	1,088.6	1	6
	7.83	1,140.4	0	9
Sodium sulfide (new sample)	6.62	231.6	0	10
	7.69	416.3	0	10
Sodium disulfide (old sample)	6.70	1,086.9	2	5
	7.16	1,111.9	0	9
Sodium disulfide (new sample)	6.70	602.3	1	8
	7.61	986.1	1	6
Sodium sulfate (control)	6.78	1,188.9	0	6
	7.08	1,173.0	0	8

[1] Starch is rated from 0 (none) to 5 (very profuse).
[2] Sporulation is rated from 0 (sterile) to 10 (maximum). All spores were black.

Further insight into the mechanism of sulfide and disulfide utilization is afforded by the data of table 2. Attention is called to the increased yields accompanying increased age of the sample of sulfide. A slight initial alkalinity of the nutrient solution led in all cases to an increase in yield, particularly with the fresh and presumably unoxidized sample. This is interpreted to imply that sulfide and disulfide are more rapidly altered in alkaline solution to forms assimilable by *Aspergillus niger*. It is a matter of general knowledge to mycologists that even higher acidities than are here dealt with promote increased growth of this fungus.

Insufficient evidence is available to form a final opinion on the cause of the higher yields with disulfide as compared with sulfide. The use of sulfur washed with alcohol and water for the preparation of disulfide has little effect on yield. Sulfur is quite difficult to obtain pure and free from oxygen except by recrystallization in carbon disulfide and subsequent distillation in nitrogen (*1*). Washing the sulfur as aforesaid, prior to the preparation of disulfide, would appear of little value in this connection (*8*).

The possibility exists that chemical transformations that result in the formation of assimilable sulfur are more readily undergone by disulfide than by sulfide. Stock solutions of the former alter more rapidly on standing than do those of the sulfide (yield of fungus).

ASSIMILABILITY OF SULFUR IN CYSTINE, METHIONINE, AND THIAMIN [4]

Cysteine and oxidized derivatives (table 3) gave maximum growth when supplied as sources of sulfur. The same is true for taurine, which might be considered as formed from cysteic acid by decarboxylation, and for taurine disulfoxide. Deaminization of taurine to form ethane sulfonic acid does not diminish the assimilability of the contained sulfur, as is shown by the yields obtained with potassium ethane sulfonate. Benzylation of the sulfhydryl group of cysteine was only moderately effective in decreasing the availability of cysteine sulfur. Methionine, cystine, cysteine, and homocystine were equally effective as sources of sulfur supply. A test with thiamin chloride revealed that this essential metabolite in the nutrition of the fungi was unable to serve as a general source of sulfur supply.

TABLE 3. *Growth data for Aspergillus niger after 4 days at 35° C. with metabolites or their derivatives providing 25 mg. of sulfur per liter of solution*

Source of sulfur	Formula	Yield per 2.5 gm. of sucrose	Sporulation [1]
		Mg	
No sulfur (control)		19.1	2
Cysteic acid	$CH_2(SO_3H)\cdot CH(NH_2)\cdot COOH$	1,110.7	8
Cysteine sulfinic acid	$CH_2(SO_2H)\cdot CH(NH_2)COOH$	1,239.0	8
Cysteine disulfoxide	$(C_3H_6O_2N)SO\cdot S(C_3H_6O_2N)$	1,161.5	8
l Cystine	$[-S-CH_2CH(NH_2)COOH]$	1,218.9	8
Cysteine hydrochloride	$HSCH_2\cdot CH(NH_2\cdot HCl)\cdot COOH$	1,087.3	8
Taurine	$NH_2CH_2CH_2SO_3H$	1,039.5	8
Taurine disulfoxide	$NH_2CH_2CH_2SO_2\cdot SCH_2CH_2NH_2$	1,126.9	8
Potassium ethane sulfonate	$C_2H_5SO_3K$	1,176.9	8
dl Methionine	$CH_3SCH_2CH_2CH(NH_2)COOH$	1,183.2	8
Homocystine	$[-SCH_2CH_2CH(NH_2)COOH]_2$	1,103.8	1
S benzylcysteine	$C_6H_5CH_2\cdot SCH_2CH(NH_2)COOH$	623.3	8
Thiamin chloride [2]	$C_{12}H_{17}N_4SOCl$	80.5	1

[1] Sporulation is rated as 0 (sterile) to 10 (maximum). All spores were black.

Only 22.2 mg. S/L.

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[4] Lanthionine $[(HO_2C\cdot CH(NH_2)CH_2)_2S]$ and "optically inactive cystine" were obtained, subsequent to completion of this manuscript, from Dr. D. B. Jones and Dr. M. J. Horn, of the Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture. The yield with this new amino acid was only 159.3 mg., while that with "optically inactive cystine" was 1,050.2 mg., or about that obtained with *l* cystine.

The state of oxidation of sulfur in anabolites is immaterial, therefore, in their use by the organism as a general source of sulfur supply. This is in marked contrast to the results previously discussed on the assimilation of inorganic sulfur. It is evident, therefore, that a clear distinction must be made in the nutrition of this fungus between compounds requiring digestion and those utilized without a preliminary alteration, because they are identical with substances (anabolites) necessarily formed during development.

ASSIMILABILITY OF SULFUR IN MISCELLANEOUS ORGANIC COMPOUNDS

Growth data for *Aspergillus niger* with sulfur compounds of the mercaptan-sulfonate series, the sulfide-sulfone series, and miscellaneous compounds are given in table 4. The slight increases in yield with *n*-propyl sulfone, *n*-amyl disulfide, benzyl isothiourea, and benzoyl persulfide are perhaps fortuitous and due to impurities of other sulfur compounds. An interesting feature of these responses is the failure of ethyl, propyl and heptyl mercaptans to serve as sources of sulfur. It can probably be assumed that all alkyl mercaptans are useless as sulfur supply. Nevertheless ethane sulfonic acid, an oxidation product of ethyl mercaptan, furnishes sulfur in readily available form, as does isoamyl sulfonic acid (table 5).

TABLE 4.—*Growth data for Aspergillus niger after [illegible] days at 35° C. with miscellaneous organic compounds providing 25 mg. or more of sulfur per liter of solution*

Source of sulfur	Formula	Yield per 5 gm. of sucrose	Sporulation[1]
		Mg.	
No sulfur (control)		4.7	0
Dimethyl sulfone	$(CH_3)_2SO_2$	6.7	1
Ethyl mercaptan	C_2H_5SH	3.1	0
n-Propyl mercaptan	C_3H_7SH	6.3	0
n-Propyl sulfide	$(C_3H_7)_2S$	6.8	0
n-Propyl sulfone	$(C_3H_7)_2SO_2$	213.8	8
n-Propyl disulfide	$C_3H_7SSC_3H_7$	3.7	0
n-Heptyl mercaptan	$C_7H_{15}SH$		0
n-Heptyl sulfide	$(C_7H_{15})_2S$	6.4	0
n-Amyl disulfide	$(C_5H_{11}S)_2$	211.2	1
Sodium hydroxymethane sulfinate	$HOCH_2SO_2Na$	1,195.8	9
Thioglycolic acid	$HSCH_2COOH$	0	0
Potassium ethyl xanthate	$C_2H_5OCS_2K$	0	0
Benzyl isothiourea hydrochloride	$C_6H_5CH_2SC(NH_2)NH$	12.4	8
Di(β-phenylpropiophenone) β-sulfide	$[C_6H_5CH(CH_2COC_6H_5)]_2S$	13.1	1
Thiourea	$(NH_2)_2CS$	7.6	0
Sulfonal	$(CH_3)_2C(SO_2C_2H_5)_2$	6.2	0
Diphenyl thiocarbazone	$C_6H_5NNCSNHNHC_6H_5$	23	2
Sodium benzene sulfinate	$C_6H_5SO_2Na$	23	2
Diphenyl sulfone	$(C_6H_5)_2SO_2$	17.3	2
Diphenyl disulfide	$C_6H_5SSC_6H_5$	0	0
Diphenyl sulfoxide	$(C_6H_5)_2SO$	16.4	2
Benzoyl persulfide	$(C_6H_5CO)_2S_2$	188.7	1
Benzyl disulfide	$(C_6H_5CH_2S)_2$	2.1	0
Benzyl sulfide	$(C_6H_5CH_2)_2S$	0	0
Trithiomethylene	$(H_2CS)_3$	2.6	0
Thioacetic acid	CH_3COSH	0	0
Thioacetamide	CH_3CSNH_2	600.7	2
β,β-Dihydroxyethyl sulfide	$(HOCH_2CH_2)_2S$	400.8	3
Potassium dithiooxalate	$(COSK)_2$	730.7	1

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[1] Sporulation is rated from 0 (sterile) to 10 (maximum). All spores were black.

TABLE 5. *Effect of barium ion on growth of Aspergillus niger (4 days at 35° C.) with various sulfur compounds*

Source of sulfur (25 mg. of sulfur per liter)	Yield per 2.5 gm. of sucrose	
	No barium added	Barium added (120 mg. per liter)
	Mg.	*Mg.*
Sodium sulfate	1,209.7	414.8
Taurine	1,190.4	1,024.1
Cysteic acid	1,147.1	1,131.9
Potassium ethane sulfonate	1,216.4	1,204.3
Sodium iodomethane sulfonate	116.6	83.7
Sodium isoamyl sulfonate	1,183.0	885.7
Sodium 2-bromethane sulfonate	40.0	35.5
Thioacetamide	481.0	133.1
β, β Dihydroxyethyl sulfide	568.2	475.5
Potassium dithiooxalate	463.2	451.0

Trial of alkyl sulfinates could not be made, but growth with sodium hydroxymethane sulfinate (sodium formaldehyde sulfoxylate) was fully equal to that of any source of sulfur. Its sulfur is in the lowest stage of oxidation compatible with maximum efficiency of assimilation of all organic compounds investigated except methionine, cysteine, homocystine, and cystine. It is important to note that, like all sulfinates, it may be considered a derivative of sulfoxylic acid, whose sulfur is in the lowest stage of oxidation for maximum efficiency of assimilation of all inorganic compounds investigated. That is to say, sulfoxylate and sulfinate are synonymous terms, the one used for inorganic compounds, the other for organic. The chemistry of sodium hydroxymethane sulfinate has been discussed by Bazlen (*3*) and Whitmore (*20*).

The difference in nutritive value of sulfur in sulfhydryl and disulfide anabolites (cysteine, cystine, and methionine) and of catabolites (alkyl mercaptans, alkyl disulfides) is sharp and clean-cut. It would indicate that the important factor, at least in metabolism, is molecular configuration as a whole, and not the configuration of specific groups in the molecule.

Other interesting features are nonassimilability of benzene sulfur derivatives, the availability of sulfur in dithiooxalic acid in contrast to the unavailability of that in thioacetic acid, the effect of an amino group attached to the same carbon to which sulfur is attached by a double bond (thioacetamide), and the effect of introduction of oxygen into the alkyl groups of ethyl sulfide (β, β-dihydroxyethyl sulfide). These citations furnish examples of the influence of adjacent groups in the organic molecule upon the rate and ease of assimilation of its contained sulfur.

Availability of a number of alkyl sulfonates permitted a closer experimental study of the method whereby they are utilized as a source of sulfur by the fungus. Several possibilities exist. Sulfur may be used only after hydrolytic removal from the molecule as free sulfonic, sulfinic, or sulfenic acid, depending on concomitant reduction. The nature of the alkyl group would be of little influence in this event. If the barium ion is without effect upon sulfur utilization, it can be assumed that its hydrolytic removal does not occur as the sulfate (*16*).

If the composition of the alkyl residue does determine the extent of sulfur assimilation, the possibility exists that sulfur is assimilated only in combination with a specific carbon group, or that the presence of substituents may prevent nonhydrolytic removal of sulfur.

Examination of table 5 discloses that a considerable difference in composition of the alkyl group [CH_3CH_2 - as compared with $(CH_3)_2CH_2CH_2CH_2$] had no influence on the efficiency of sulfur utilization. However, the presence of halogen substituents in sodium iodomethane sulfonate and sodium 2-bromoethane sulfonate definitely prevented the assimilation of sulfur. It is improbable that toxicity plays a part in this response, inasmuch as the former compound is claimed to be inert in the animal after ingestion and suitable for X-ray diagnoses. The possibility is slight that the presence of halogen prevents the hydrolytic removal of sulfur, since in compounds such as trichloracetic acid and trichloracetaldehyde its effect is to weaken the carbon-to-carbon bond. It seems likely, therefore, that cleavage of sulfur from these compounds by the organism is nonhydrolytic, a residue with a double bond being formed.

Several reasons render probable the interpretation that the sulfur is split off in the form of free sulfoxylic acid (HSO_2H). The ineffectiveness of the presence of barium ion would indicate that sulfur is not removed hydrolytically or otherwise as sulfuric acid. Its removal as free sulfurous acid (HSO_3H) is possible, of course, but improbable from the chemical point of view. Lastly, alkyl sulfinate is equivalent to sulfate-sulfur in assimilability and may undergo this type of cleavage with production of free sulfinic acid (HSO_2H). Whether, however, sulfur is reduced still further before cleavage to free sulfenic acid ($HSOH$) must await further investigation. As far as the evidence goes, the indications are that sulfur is removed from organic compounds as free sulfinic acid, and that the process is probably enzymatic and due to the presence of "desulfinase."

Since the splitting off of the sulfonic acid group is doubtful under these conditions from what is known of its chemistry, the evidence would indicate that reaction occurs only after reduction at least to sulfinate has occurred. This conclusion is identical with that arrived at on the basis of the data on assimilation of inorganic sulfur and on that of sodium hydroxymethane sulfinate.

EFFECTS OF TRACE-ELEMENT DEFICIENCIES WITH VARIOUS SULFUR COMPOUNDS

Further data on the relation of inorganic sulfide and disulfide to growth of *Aspergillus* are brought out in table 6. The yields would indicate that age of sample and alkalinity of the nutrient solution favor the rapid transformation of sulfide and disulfide into forms readily assimilable by the fungus. The addition of sodium disulfide to the nutrient solution invariably causes the formation of a profuse precipitate, presumably of finely divided sulfur. This precipitate largely disappeared during the course of growth of the fungus. A precipitate is also formed with sodium thiosulfate in acid solution and disappears similarly.

TABLE 6 *Effect of age of sulfide and disulfide sulfur on growth of Aspergillus niger for 4 days at 35° C and on the responses of the fungus to deficiencies in trace elements*

Element omitted	Sodium sulfide (old sample)					Sodium disulfide (old sample)					Sodium sulfide (fresh sample)					Sodium disulfide (fresh sample)					Sodium sulfide (fresh sample in non-alkaline solution)				
	Yield per 2.5 gm of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]	Yield per 2.5 gm of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]	Yield per 2.5 gm of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]	Yield per 2.5 gm of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]	Yield per 2.5 gm of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]
	Mg	*Pct*	*pH*			*Mg*	*Pct*	*pH*			*Mg*	*Pct*	*pH*			*Mg*	*Pct*	*pH*			*Mg*	*Pct*	*pH*		
None	1,140.4	100.00	1.85	0	9	1,111.9	100.00	1.86	0	9	446.3	100.00	2.01	0	10	986.1	100.00	1.88	1	6	359.0	100.00	2.14	0	10
Fe	161.2	14.14	2.00	0	4	172.4	15.51	1.99	0	4	102.1	22.88	2.23	0	4	88.5	8.98	2.26	0	2	232.7	64.82	1.99	0	10
Zn	416.7	36.54	1.79	0	10	414.0	37.29	1.81	0	10	310.1	69.48	2.02	0	10	242.0	24.54	1.96	0	10	290.2	80.82	2.14	0	10
Cu	1,154.9	101.27	1.95	0	6 br	1,144.4	102.97	2.05	0	6 t	464.4	104.05	1.85	0	8	892.2	90.48	2.07	1	2	293.4	81.71	2.04	0	10
Mn	651.7	57.15	1.49	3*	1	634.5	57.10	1.70	1	0	369.5	82.79	1.64	3*	2	456.3	46.27	1.64	4*	1	236.8	65.96	1.67	2	0
Mo	1,126.4	98.77	1.78	0	9	1,065.5	95.81	1.83	0	4	532.0	114.34	1.84	1	10	1,042.7	105.74	1.95	0	6	323.9	90.21	2.02	0	10
Ga	1,128.6	98.97	1.87	0	9	1,111.8	99.99	1.84	0	8	501.2	112.19	1.91	1	10	979.2	99.30	1.97	0	6	315.6	87.89	2.11	0	10
Maximum [3]	1,146.1					1,159.8					539.4					1,102.8					388.1				
C U [4]		45.84					46.39					21.58					44.11					15.52			
pH [5]			7.84					7.40					7.64					7.61					4.18		

[1] Starch is rated from 0 (none) to 5 (very profuse); an asterisk denotes the immediate formation of a blue color on addition of iodine. Starch in substrate indicated in parentheses. Erythrodextrin indicated by E.

[2] Sporulation is rated as 0 (sterile) to 10 (maximum). All spores are black unless otherwise stated: br=brown, t=tan, y=yellow, w=white.

[3] Maximum individual yield.

[4] Coefficient of utilization or yield per 100 gm of sucrose.

[5] Initial acidity of nutrient solution.

The acidities employed in the acid nutrient solution in table 6 are not considered too high, since Mellor (8) states that a solution of 0.5 N-HCl (pH = 0.36±) has been found to dissolve 2.54 times its volume of hydrogen sulfide. This is more than ample to supply the quantity of sulfur required for maximum growth. The data for sodium disulfide in acid solution have been omitted, since yields were maximum owing to complete aging of disulfide.

The sample of sulfur used in the preparation of the disulfide gave appreciable increases in growth when used as a sole source of sulfur. It is possible, therefore, that elemental sulfur in a very finely divided form is capable of utilization by the fungus in the presence of reduced inorganic sulfur compounds because it is more readily converted to assimilable sulfur chemically. Trials with equal quantities of elemental sulfur and of sulfur as sulfide, hyposulfite, sulfite, or thiosulfate gave increases over and above those attributable to the individual constituents with sulfur and thiosulfate and with sulfur and hyposulfite.

Results with trace elements proved disappointing in view of the insolubility of sulfides (except gallium). The marked acidification of the substrate during the course of development was probably responsible for the negative results obtained. As previously mentioned, ferrous sulfide readily dissolves under these conditions. Nevertheless, cupric sulfide is insoluble at much higher acidities than were here encountered. The data would indicate, however, that the usual trace elements were required for growth. Deficiency of individual trace elements was without marked effect on acidity of the cultures at harvest, or on starch in the mycelial felts, except in those cases in which addition of manganese was omitted.

The effects of a trace-element deficiency with various sulfur compounds serving as sources of sulfur in the nutrition of *Aspergillus niger* are tabulated in table 7. It is evident that the trace elements required with sulfate sulfur are also needed for growth with other inorganic sources of sulfur, no matter in what stage of reduction. A similar condition was found to exist with cystine, taurine, methionine, and their derivatives. No evidence was obtained that reduction of inorganic sulfur to a form suitable for conversion into organic sulfur is specifically associated with the presence of one of the essential trace elements. It should be realized, on the other hand, that further refinement of experimental technique may lead to quite different results in the future. Results on sporulation, formation of starch, and acidity of the substrate at harvest were also much the same with all sources of sulfur.

Interpretation of the data with propyl sulfone requires further study, though growth with this compound has been previously assumed to be due to other sulfur impurities. Dimethyl sulfone cannot supply assimilable sulfur. Nor were alkyl sulfoxides available for trial. The fact that propyl sulfide cannot be utilized should not be considered as confirmatory evidence, since ethyl mercaptan cannot be assimilated, whereas its oxidation product, ethyl sulfonate, can be used.

TABLE 7.—Effects of trace-element deficiencies on growth of Aspergillus niger for [illegible] days at 35° C., with various sources of sulfur supply[1]

Element omitted	Magnesium sulfate					Sodium thiosulfate					Sodium bisulfite					Taurine					Potassium ethane sulfonate					Cysteine hydrochloride				
	Yield per 25 gm of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 25 gm of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 25 gm of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 25 gm of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 25 gm of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 25 gm of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation
	Mg	*P*	*pH*			*Mg*	*Pct*	*pH*			*Mg*	*Pct*	*pH*			*Mg*	*Pct*	*pH*			*Mg*	*Pct*	*pH*			*Mg*	*Pct*	*pH*		
None	1 041 7	100 00	3 14		[illegible]	[illegible]	[illegible]	2 [illegible]	4	4	1 [illegible] 2	100 00	2 7	[illegible]	[illegible]	1 0[illegible]	100 00	[illegible]	4	[illegible]	1 0[illegible]3	100 00	2 41	3	[illegible]	1 00[illegible] 1	100 00	2 41	4	8
Fe	[illegible]	[illegible] 1	2 4	[illegible]	1	[illegible]	[illegible] 1	[illegible]	[illegible]	[illegible]	1 4	[illegible] 1	[illegible]	0	1	1[illegible] 0	[illegible]	2 11	1	4	100 2	9 74	2 19	0	1	141 2	14 03	2 24	1	4
Zn	134 9	12 [illegible]	2 35	0	1	30[illegible]	[illegible]	[illegible]	0	1	1	4	2 [illegible]		1	[illegible]	2 22	2 9[illegible]	0	1	[illegible]2 8	16	2 [illegible]	[illegible]	1	41 1	4 0[illegible]	2 7	1	1
Cu	931 9	89 46	3 14	4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1	742	72	[illegible]		1	[illegible]	[illegible]	2 2	4	[illegible]	81[illegible]	[illegible]	2 47	[illegible]	1	954 [illegible]	[illegible]4 94	[illegible] 47	2	2
Mn	976	[illegible] 44	1 98	4	[illegible]	812	[illegible]	2 0[illegible]	[illegible]		1 0[illegible]	10[illegible]	[illegible]	4	1	944 1	9[illegible]	2 02	4	1	1 10[illegible] 4	1[illegible] 0	2 [illegible]	4	2	1 0[illegible] 3	10[illegible] 07	2 0[illegible]	[illegible]	2
Mo	[illegible] 0	[illegible] 24	2 [illegible]	4	3	82	[illegible]	[illegible] 34		4	813	[illegible]	1		[illegible]	[illegible]	[illegible] 04	2 09	4	4	981 [illegible]	[illegible] 9	2 3[illegible]	4	6	9[illegible]0 [illegible]	94 77	2 19	4	6
Ga	972 3	93 53	[illegible]	3	[illegible]	82	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] 1	[illegible]	[illegible] 2		5	[illegible] 4	92 04	2 13	4	4	984 1	[illegible] 11	[illegible] 1	5	[illegible]	973 9	9[illegible] [illegible]7	2 31	4	7
Maximum	1 0[illegible] 0					1 [illegible]					1 [illegible]					1 0[illegible] 9					1 100 4					1 087 3				
C L		43 2[illegible]					41					4 [illegible]					41 [illegible]					44 [illegible]					43 49		
pH			7 [illegible]					[illegible] 28					[illegible] 1					[illegible] 4					[illegible] 10					[illegible] 40		

[1] See footnotes to table [illegible].

TABLE 7.—*Effects of trace-element deficiencies on growth of Aspergillus niger for 4 days at 35° C., with various sources of sulfur supply*—Continued

Element omitted	dl-Methionine					n-Propyl sulfone					Cysteine disulfoxide					Cysteic acid					Sodium hyposulfite					Sodium formaldehyde sulfoxylate (sodium hydroxymethane sulfinate)				
	Yield per 2.5 gm of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation
	Mg	*Pct*	*pH*			*Mg*	*Pct*	*pH*			*Mg*	*Pct*	*pH*			*Mg*	*Pct*	*pH*			*Mg*	*Pct*	*pH*			*Mg*	*Pct*	*pH*		
None	832 [illegible]	100 00	2 16		3	243 [illegible]	100 0[illegible]	[illegible] 19	[illegible]	1[illegible]	[illegible]8 2	100 00	2 2		2	1 020 [illegible]	100 00	1 [illegible]	0	[illegible]	1 082 [illegible]	100 00	3 10	2	9	1 1[illegible]3 [illegible]	100 00	1 [illegible]3	1	[illegible]
Fe	1[illegible]4	1 [illegible]	2 1		[illegible]	1 [illegible] 4	[illegible]4 [illegible]	[illegible] 2[illegible]	[illegible]	1	3 4	[illegible] [illegible]2	2 4[illegible]	[illegible]	1	2[illegible]7	2[illegible] 1[illegible]	1 94	[illegible]	[illegible]	11[illegible] [illegible]	11 [illegible]1	2 13	[illegible]	[illegible]	343 [illegible]	29 0[illegible]	1 [illegible]4	[illegible]	[illegible]
Zn	41 [illegible]	4 [illegible]	2 [illegible]		4	1 1[illegible] 4	4[illegible] 42	[illegible]	[illegible]	[illegible]	1[illegible]9 [illegible]	2[illegible] 4	2 [illegible]	4	1	[illegible]6 [illegible]	2 [illegible]1	2 [illegible]9	[illegible]	2	129 1	11 92	2 [illegible]2		1	4[illegible] [illegible]	41 14	1 [illegible]4	[illegible]	10
Cu	[illegible]40 [illegible]	[illegible] 1	[illegible]	F		2[illegible] 21[illegible] 6	[illegible]4 2[illegible]	2 14	0	1	[illegible]1 [illegible]	[illegible]4 [illegible]	2 3[illegible]4	1	0	1 0[illegible]9 [illegible]	1 4 [illegible]	[illegible]1	0	4	[illegible]13 1	7 1	2 [illegible]1	3	2	1 17[illegible] 1	[illegible]9 42	1 [illegible]7	1	4
Mn	912 1	9[illegible] [illegible]9	1 [illegible]		4	4 [illegible]43 1	99 [illegible]3	2 11	2 1	1	1 082 5	134 [illegible]4	2 1[illegible]	2	[illegible]	[illegible]	[illegible] 4[illegible]	1 [illegible]2	0	4	1 0[illegible]9 1	9[illegible] [illegible]2	1 [illegible]	3	0	[illegible]2 [illegible]	4[illegible] 9[illegible]	1 [illegible]	[illegible]	[illegible]
Mo	70[illegible] [illegible]	[illegible] 2	1 9[illegible]			4 2[illegible]1	111 3[illegible]	1 9	2 1	1[illegible]	[illegible]9	[illegible]1 [illegible]3	1 [illegible]		0	1 012	[illegible] 1[illegible]	1 [illegible]	[illegible]	[illegible]	[illegible]9[illegible] 3	64 [illegible]	1 91	[illegible]	3	1 [illegible]44 2	[illegible] 2[illegible]	1 62	1	4
Ga	[illegible]	95 [illegible]	2 1		[illegible]	[illegible] 1[illegible]	[illegible] 44	2 2	1	[illegible]	[illegible]2 3	9 [illegible]	2 [illegible]2		1	1 10[illegible] [illegible]	10[illegible] [illegible]2	1 94	0	[illegible]	1 029 2	9 0[illegible]	3 01	2	[illegible]	1 19[illegible] 0	101 23	1 [illegible]4	0	[illegible]
Maximum	963 0					2[illegible]7 [illegible]					1 082					1 140					1 104					1 2[illegible]9 [illegible]				
C I		3[illegible] 2					1[illegible] [illegible]					43 2[illegible]					4[illegible] [illegible]3					44 1[illegible]					4[illegible] 03			
pH			[illegible]					[illegible]					[illegible]4					[illegible]					[illegible]					7 39		

EFFECTS OF NUTRIENT-SOLUTION PURIFICATION WITH VARIOUS SULFUR SOURCES

Results with trace-element deficiencies after nutrient-solution purification with calcium carbonate (table 8) emphasize the results obtained with unpurified solutions. There would seem little doubt that all the essential trace elements are required irrespective of the source of sulfur. Results with iron and zinc deficiencies were good from the experimental point of view with every sulfur compound tested. Omission of copper led to the greatest decrease in growth with sodium hydroxymethane sulfinate. Best results on manganese were obtained with methionine. Cysteic acid gave maximum decreases in yield on omission of molybdenum and of gallium, but the results were poor. Little weight is placed on the data for *n*-propyl sulfone. Interpretation of these results, however, must await further evidence that they are not fortuitous.

Increased mycelial deposition of starch occurred most frequently with a deficiency in manganese, sometimes with a deficiency in molybdenum, and more rarely with a deficiency in copper or iron. This phenomenon was usually accompanied by specially high increases in acidity of the substrate and a sharp diminution in sporulation. In no instance did similar responses accompany the omission of zinc. Since yields on omission of zinc were far less than on omission of copper, manganese, or molybdenum, these differences may be only concentration effects and not specific.

DISCUSSION OF RESULTS

The growth data on sulfur utilization with compounds of the sulfide-sulfate series and the mercaptan-sulfonate series would appear to justify the assumption that reduction is the normal preliminary process in the utilization of sulfur compounds. This has been assumed in the case of sulfate assimilation (*2*, *4*, *12*). These data would indicate, however, that reduction may be carried too far. When carried beyond a certain stage (sulfoxylate or sulfinate) the nutritive value of sulfur compounds disappears. Evidently the reverse process of oxidation of sulfur is performed by the fungus with great difficulty, if at all.

A distinction should be made in this connection as concerns metabolites since the organism was able to assimilate all cystine derivatives regardless of the state of oxidation of the contained sulfur. Metabolites, or their derivatives, would appear to follow a definite metabolic channel whether synthesized by the fungus or added to the solution as a nutrient. It is possible that all assimilated sulfur passes through the amino acid stage, since cystine and methionine afford excellent sources of sulfur for general use in metabolism, equal to that of sulfate-sulfur or any other. However, it is not definitely known whether cystine and methionine are assimilated as a whole when fed to the fungus, though this is assumed to be the case. Thiamin chloride was practically unavailable as a general source of sulfur. The fungus does not suffer from the inability of mammalian forms to convert cystine into methionine.

TABLE 8.—*Effects of trace-element deficiencies on growth of Aspergillus niger for 4 days at 35° C. with various sources of sulfur supply after purification of the nutrient solution with calcium carbonate* [1]

Element omitted	Magnesium sulfate					Sodium ethyl sulfate					Sodium bisulfite					Taurine					Potassium methanesulfonate					Cystine hydrochloride				
	Yield per 25 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 25 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 25 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 25 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 25 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 25 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation
	Mg.	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*		
None	1,212.6	100.00	[illegible]	[illegible]	10	1,13[illegible]	100.0[illegible]	2.2[illegible]	0		1,2[illegible]2	100.00	1.9[illegible]	0		[illegible]32	100.00	1.91	2	[illegible]	1,22[illegible].9	100.00	2.04	0	[illegible]	1,30[illegible].3	100.00	2.9[illegible]	0	10
Fe	3.2	[illegible]	3.7[illegible]	0	0	[illegible].0	4.4	3.2	0	1	3.2	[illegible]	3.[illegible]	[illegible]	0	0.0	0	[illegible].2[illegible]	[illegible]	[illegible]	[illegible].1	4[illegible]	3.[illegible]	0	0	15.0	1.38	2.5[illegible]	0	1
Zn	[illegible]	[illegible].24	2.6[illegible]	0	[illegible]	1[illegible]	1.1	3.0[illegible]	0	1	21.[illegible]	1.[illegible]	2.[illegible]	[illegible]	2	24	2.44	3.01	0	1	1[illegible].5	1.40	2.[illegible]	0	1	33.8	2.59	2.53	0	2
Cu	422	[illegible]	2.[illegible]4	2	2	1,1[illegible]3	[illegible]3.9[illegible]	2.[illegible]1	[illegible]	0	1,20[illegible]	[illegible]4	2.1[illegible]	0	4	3[illegible]2.3	43.2	1.[illegible]	1	4	9[illegible].4	4[illegible].21	1.72	2	2	[illegible].4	[illegible].76	2.2[illegible]	0	0
Mn	[illegible]3.0	4[illegible]	1.[illegible]	*4	0	430.1	[illegible]1.1	[illegible]4	2	0	4[illegible]2.[illegible]	3[illegible]	1.[illegible]	*	0	3[illegible]2	4[illegible].3	1.[illegible]	*2	[illegible]	441.3	3[illegible].22	1.[illegible]	[illegible]*[illegible]3	0	[illegible]9.1	41.30	1.8[illegible]	[illegible]*	0
Mo	1,1[illegible]4.3	9[illegible].[illegible]4	2.2[illegible]	0	4	1,222.[illegible]	[illegible]0.13	1.[illegible]3	0	[illegible]	1,13[illegible].8	[illegible].3[illegible]	1.[illegible]	[illegible]	2	[illegible]	[illegible]1.1	1.61		[illegible]	1,180.4	94.21	1.9[illegible]	0	4	1,172.2	[illegible]9.[illegible]0	1.[illegible]	4	4
Ga	1,[illegible]	104.41	2	0	10	1,33[illegible]	[illegible]8.20	2.40	0	[illegible]	1,24[illegible].2	[illegible]	2.0[illegible]	[illegible]	[illegible]	[illegible]4[illegible]	[illegible]	1.[illegible]	—	[illegible]	1,271.[illegible]	101.4[illegible]	2.2[illegible]	0	[illegible]	1,33[illegible].6	102.40	2.74	0	[illegible]
Maximum	1,266.0					1,3[illegible]2.0					1,3[illegible]0.2					[illegible]					1,3[illegible]					1,33[illegible].2				
C. V.		[illegible]4					4.1[illegible]					2.[illegible]					2					3.11					53.53			
pH			1.9					[illegible]					[illegible]					[illegible].24					[illegible].41					7.07		

[1] See footnote to table [illegible].

TABLE 8.—*Effects of trace-element deficiencies on growth of Aspergillus niger for 4 days at 35° C. with various sources of sulfur supply after purification of the nutrient solution with calcium carbonate*—Continued

Element omitted	dl-Methionine: Yield per 25 gm. of sucrose	dl-Methionine: Proportion of maximum yield	dl-Methionine: Acidity at harvest	dl-Methionine: Starch in mycelium	dl-Methionine: Sporulation	n-Propyl sulfide: Yield per 25 gm. of sucrose	n-Propyl sulfide: Proportion of maximum yield	n-Propyl sulfide: Acidity at harvest	n-Propyl sulfide: Starch in mycelium	n-Propyl sulfide: Sporulation	Cysteine hydrochloride: Yield per 25 gm. of sucrose	Cysteine hydrochloride: Proportion of maximum yield	Cysteine hydrochloride: Acidity at harvest	Cysteine hydrochloride: Starch in mycelium	Cysteine hydrochloride: Sporulation	Cysteic acid: Yield per 25 gm. of sucrose	Cysteic acid: Proportion of maximum yield	Cysteic acid: Acidity at harvest	Cysteic acid: Starch in mycelium	Cysteic acid: Sporulation	Sodium hyposulfite: Yield per 25 gm. of sucrose	Sodium hyposulfite: Proportion of maximum yield	Sodium hyposulfite: Acidity at harvest	Sodium hyposulfite: Starch in mycelium	Sodium hyposulfite: Sporulation	Sodium formaldehyde sulfoxylate (sodium hydroxymethane sulfinate): Yield per 25 gm. of sucrose	Sodium formaldehyde sulfoxylate (sodium hydroxymethane sulfinate): Proportion of maximum yield	Sodium formaldehyde sulfoxylate (sodium hydroxymethane sulfinate): Acidity at harvest	Sodium formaldehyde sulfoxylate (sodium hydroxymethane sulfinate): Starch in mycelium	Sodium formaldehyde sulfoxylate (sodium hydroxymethane sulfinate): Sporulation
	Mg.	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*		
None	1,109.6	100.00	1.84	1	[illegible]	[illegible]4	100.00	3.01	0	4	1,22[illegible].[illegible]	100.00	2.00	0	[illegible]	672.9	100.00	1.74	0	8	1,193.5	100.00	2.08	0	8	1,281.7	100.00	2.25	1	6
Fe	0	0	[illegible].37	1	0	[illegible]	0	6.11	[illegible]	0	[illegible].1	.2	3.48	0	0	5.6	.83	[illegible].32	0	1	[illegible]6	.13	3.04	0	2	18.3	1.42	2.97	0	1
Zn	28.3	2.[illegible]1	2.87	0	4	1.0	2.[illegible]2	[illegible].18	[illegible]	4	[illegible]4	2.47	2.40	0	3	17.3	2.58	3.02	0	2	16.3	1.25	2.93	0	2	5.7	.45	3.00	0	0
Cu	1,146.0	113.[illegible]	2.16	0	4	28.4	2.[illegible]	[illegible].03	0	4	1,217.[illegible]	[illegible]9.03	[illegible].32	0	3	[illegible]50.0	74.4	1.74	0	8	750.[illegible]	57.[illegible]	1.78	2	4, br	4[illegible]6.5	36.40	1.67	5* (3)	0
Mn	225.8	22.[illegible]	1.78	[illegible]	0	28.3	1.[illegible]3	2.[illegible]7	0	4	33[illegible].[illegible]	27.37	1.12	5* 2	0	261.1	38.81	1.68	5* 0	0	373.[illegible]	28.66	1.55	5* (3	0	523.9	40.[illegible]	1.53	5* (3)	0
Mo	[illegible]20.7	[illegible]1.94	1.60	5* ([illegible]	[illegible]	44.1	80.33	2.91	0	4	9[illegible].2	[illegible]7.24	1.70	[illegible]	4	544.8	80.[illegible]	1.61	0 2	8	1,267.0	9[illegible].20	1.96	0	6	1,189.7	92.82	1.99	1	4
Ga	1,041.6	103.17	1.95	0	6	38.3	60.[illegible]	2.94	0	4	1,2[illegible].2	102.38	2.19	0	[illegible]	555.2	82.[illegible]1	1.[illegible]1	0	8	1,315.[illegible]	101.15	2.16	0	8	1,264.6	99.67	2.26	1	6
Maximum	1,14[illegible].0					71.2					1,2[illegible].2					834.8					1,301.[illegible]					1,326.2				
C. V.		4[illegible].54					2.[illegible]					[illegible]					35.3[illegible]					54.46					53.95			
pH			7.00					7.23					7.1[illegible]					7.01					7.08					7.8[illegible]		

If metabolites serving as nutrients may be pictured as passing into the normal channels present in the organism for their use, the case of nonmetabolites falls into a different category. Nutrients must first be altered to a form suitable for assimilation (digestion) and so brought into normal metabolic channels.

While mercaptans are formed by various organisms, they are probably products of catabolism (waste products) and therefore without a normal channel for anabolism. In this case also, sulfur as alkyl mercaptan was unassimilable, whereas it became readily assimilable after oxidation to alkyl sulfonate. Possibly alkyl sulfinates[5] (RSO_2H) or alkyl sulfinates ($RSOH$) will be found equally effective. It appears probable, therefore, that with organic compounds also a basic distinction exists in the absence of oxygen and its presence in combination with sulfur, or in an adjacent group. The partial utilization of substances like β, β-dihydroxyethyl sulfide, whereas ethyl sulfide presumably cannot supply sulfur, illustrates the point under discussion.

Though sulfur in alkyl sulfones is combined with oxygen, these proved to be poor sources of sulfur supply. The reason for this may be the inability of the fungus to effect their reduction. It is known that even nascent hydrogen cannot bring about their reduction. Investigation of the assimilability of sulfur in alkyl sulfoxides should help to decide whether this is the correct or sole interpretation.

Other factors of molecular configuration may prevent the utilization of organic sulfur. Sulfur attached to the benzene ring was unsuitable for use in assimilation irrespective of its state of oxidation. However, further tests may reveal that the presence of adjacent groups may considerably modify this response. Sulfur in the thiazole ring (thiamin chloride), or other rings presumably, will probably prove of little value as a nutrient, unless special conditions exist that permit of its ready release in combination with oxygen. The fungus seems unable to avail itself of sulfur attached to carbon, as in thiourea, though again exceptions may exist. The number of sulfur derivatives available for test was far too small to arrive at final conclusions. There is some indication that the presence of adjacent groups ($HO-$, CO , NH_2) may increase the availability of sulfur.

Nevertheless, the different lines of approach employed in the study of sulfur assimilation by *Aspergillus niger* have yielded an identical conclusion. Sulfur, it was found, was reduced and transformed to sulfoxylic acid when supplied as sulfate or alkyl sulfonate. Hydroxymethane sulfinate, a derivative of sulfoxylic acid, was as effective as sulfate in supplying sulfur. Miscellaneous compounds effective as sources of sulfur supply seem to require the presence of oxygen in the molecule. A further reduction to sulfenate before assimilation is a theoretical possibility, though no alkyl sulfenates are known. Whether other available sulfur compounds (β, β-dihydroxyethyl sulfide, etc.) also decompose to form sulfinate is unknown and must await further investigation. It should not be overlooked, however, that anabolites need not conform to this behavior.

Nicolet (*11*) has suggested the possibility of methylene pyruvic acid being a precursor in the utilization by the plant of sulfur in the form of inorganic sulfide or disulfide or as mercaptan, to account for the formation of methionine. Experimental results with *Aspergillus niger* would indicate, however, that the forms of sulfur postulated by Nicolet are unassimilable by this fungus. Other organisms (bacteria) may be capable of assimilating sulfide, disulfide, and mercaptan and may possibly follow the Nicolet course of assimilation. A clear distinction should be made by investigators of this question between utilization for energy and as units for essential metabolites. Experimental proof with aerobic forms would be difficult or impossible because of atmospheric oxidation. Conditions with anaerobes would probably be such as to afford adequate proof.

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[5] A supply of sodium *n* butane sulfinate ($CH_3CH_2CH_2CH_2SO_2Na$) became available through the kindness of Dr. William H Ziegler and Dr Ralph Connor, of the John Harrison Laboratory, University of Pennsylvania, Philadelphia, Pa , after completion of this manuscript Yield with 25 mg. per liter of sulfur supplied as this compound was 1,173 2 mg In the presence of barium ion (see table 5) the yield was 768 0 mg

Nonassimilation of sulfur in sulfide, disulfide, and alkyl mercaptan by *Aspergillus* does not necessarily imply that methylene pyruvic acid does not serve with this organism as a precursor of methionine. The available information might readily be interpreted on the basis of side reactions that lead to the locking of sulfur in unavailable form. Addition of sulfoxylic acid at the double bond of methylene pyruvic acid is quite probable chemically. Other biological data would indicate that forms of sulfur (sulfide, thiourea, etc.) unavailable to *Aspergillus niger* are readily assimilated by certain of the bacteria.

SUMMARY

Alterations in the source of sulfur supply were practically without effect on the trace-element requirements of *Aspergillus niger* Van Tiegh. Iron, zinc, copper, manganese, molybdenum, and gallium were apparently required in approximately equal degree, whatever the state of oxidation of sulfur supplied as a nutrient. The slightly better results obtained through omission of copper with sodium hydroxymethane sulfinate, of manganese with methionine, and of molybdenum and gallium with cysteic acid may prove to have been due to chance.

A survey of the assimilability of inorganic sulfur compounds indicated that sulfur is reduced to sulfoxylate prior to its conversion to organic sulfur. Sulfide and disulfide were not assimilated.

Assimilability of organic sulfur varied with molecular configuration and was also correlated with the presence of attached or adjacent oxygen in the molecule. Alkyl mercaptans, sulfides, and disulfides could not be used as a source of sulfur, whereas alkyl sulfonate and alkyl sulfinate were readily available. Utilization of sulfur in alkyl sulfonates and alkyl sulfinates was considered to depend on their decomposition into free sulfinic acid and an unsaturated residue.

Anabolites, particularly cystine and its derivatives, homocystine, and methionine, were readily available as sole sources of sulfur supply, irrespective of the state of oxidation of their contained sulfur, and were assumed to follow the normal channel for their metabolism. Catabolites and miscellaneous synthetic organic sulfur compounds were assumed to require a process of digestion before assimilation.

ADDENDUM

The data in table 9 were obtained after completion of the manuscript of this paper and are included as a demonstration of the assimilability of alkyl sulfinate by *Aspergillus*. The difficulty with which compounds of this type can be obtained also made it desirable to report on its utilization as fully as possible.

TABLE 9.—*Growth of Aspergillus niger at 35° C. for 4 days with sulfur supplied as sodium n-butane sulfinate*[1]

Element omitted	Unpurified (25 mg. sulfur per liter)					Purified with $CaCO_3$ (50 mg. sulfur per liter)				
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation
	Mg.	*Percent*	*pH*			*Mg.*	*Percent*	*pH*		
None	1,041.0	100.00	1.71	0	8	1,262.5	100.00	2.54	0	10
Fe	221.6	21.28	1.96	0	6	6.8	.54	2.91	0	0
Zn	379.6	36.47	1.75	0	10	8.4	.66	2.91	0	0
Cu	1,150.6	110.53	1.84	2	8	1,016.7	80.55	2.05	2	4
Mn	812.0	78.01	1.57	3	4	728.2	57.68	1.53	1	4
Mo	1,025.1	98.50	1.62	1	6	1,096.5	86.85	1.89	2	6
Ga	1,108.7	106.51	1.66	1	8	1,269.0	100.51	2.64	2	10
Maximum	1,150.6					1,295.7				
C. V.		16.02					51.83			
pH			7.35					7.47		

[1] See footnotes to table 6.

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INHERITANCE STUDIES ON DURATION OF DEVELOPMENTAL STAGES IN CROSSES WITHIN THE GENUS LYCOPERSICON[1]

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INTRODUCTION

This study deals with the inheritance of duration of three stages of development in certain crosses involving varieties of *Lycopersicon esculentum* Mill. and *L. pimpinellifolium* (Jusl.) Mill. These three stages are (1) number of days from seeding to first bloom, (2) number of days from first bloom to first fruit set, and (3) number of days from first fruit set to first complete change of color of any fruit. The sum of these three stages obviously represents the number of days from seeding to first ripe fruit and may be regarded as the measure of an earliness-of-maturity character.

The purposes of the investigation were to determine whether these several natural biological periods in the development of the tomato plant are definite subcharacters; to obtain information concerning heterosis and dominance; and to ascertain the efficiency of the fit between obtained and theoretical means, based on certain formulas (*2*, *24*, *29*),[2] as a method of determining whether the effects of the genes differentiating the quantitative characters are arithmetically or geometrically cumulative. The investigation was carried out at the Cheyenne Horticultural Field Station, Cheyenne, Wyo.

Throughout the study and the interpretation of the data, the authors have attempted to keep in mind the probability that the genes bring about the differentiation of a character by initiating (either directly or indirectly) developmental processes which, no doubt, in many cases interact among themselves (*10*, *11*, *15*, *20*, *29*, *32*, *42*, *43*).

EXPERIMENTAL CONSIDERATIONS

The design of an experiment has considerable bearing upon the interpretation of the data. Such being the case, the purpose or purposes of an experiment play a considerable part in determining the experimental design. The design for the present study was based on genetic and statistical considerations. The genetic aspects will be considered first.

From the immediately foregoing statements, it is apparent that the experimental design should allow for testing whether the stages (number of days from planting to first bloom, number of days from first bloom to first fruit set, and number of days from first fruit set to

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[2] Italic numbers in parentheses refer to Literature Cited, p. 146.

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first complete change of color of any fruit) are definite subcharacters, i. e., biologically sound subdivisions, of number of days from planting to first complete change of color of any fruit. To test the logic of such a division it is necessary to have parents that differ phenotypically as regards these stages. *Lycopersicon pimpinellifolium*, variety Red Currant, and *L. esculentum*, varieties Johannisfeuer, Danmark, and Bonny Best, were believed to have this diversity and for that reason were used in the study.

Next, the experiment should provide for measuring heterosis and dominance. Since heterosis and dominance may be determined by comparing the expression of a character in the F_1 with the expression of the same character in each of the parents, at least both parents and the F_1 should be included in the study.

Also, the design should allow for determining the efficiency of the method involving the fit between obtained and theoretical means for testing whether the effects of the genes are arithmetically or geometrically cumulative. Lindstrom (*21*), Powers (*29*), and Lyon (*23*) have pointed out the necessity, in such studies, of taking dominance into consideration. Therefore, in order to obtain the most information possible from any particular cross this method requires that both parents, the F_1 and F_2 generations, and the progenies obtained from backcrossing the F_1 to each parent be included.

One other genetic consideration in the design of the experiment should be mentioned. Since the facilities of most investigators are limited, one usually has the choice, in field investigations, between a larger sample of plant material, as represented by number of crosses studied, and a larger sample of environmental conditions, as represented by years. To obtain as large a sample of plant material as possible it would be necessary to grow different crosses every year; whereas to obtain as large a sample of years as possible, it would be necessary to grow the same crosses every year. In this study the authors were more interested in obtaining as large a sample of plant material as possible but did not want to neglect entirely environmental effects as represented by years. Consequently, the cross Johannisfeuer × Red Currant was grown in 1937 and 1939, the cross Johannisfeuer × Danmark was grown in 1938 and 1939, the cross Danmark × Red Currant was grown in 1938, and the cross Johannisfeuer × Bonny Best was grown in 1939. Furthermore, this design provided that Johannisfeuer, Red Currant, and Danmark be crossed in all combinations and that Johannisfeuer be crossed with all varieties used in the study. The sample of plant material was sufficient to allow the drawing of definite conclusions, and the sample of years was sufficient to furnish information on the interaction between environment and the genes differentiating the duration of stages of development.

The experimental design as regards the greenhouse and field technique was very similar to that reported by Powers (*28*, *29*). Consequently, only such details as are essential to an interpretation of the data will be given here. The parents and generations were grown in a randomized-block experiment, there being a total of 9 blocks in 1937 (with the exception of the F_1 generation, which will be mentioned later) and a total of 20 blocks in 1938 and again in 1939. One of the replications of the Bonny Best parent was lost in 1939. Yates' method (*44*) was used in calculating the missing plot. The material was randomized within blocks by the use of Tippett's tables (*38*), and the randomization was the same for the greenhouse and the field. The seed was sown on April 12 in 1937, on April 20, 21, and 26 in 1938, and on April 17 in 1939. The plants were spotted out in flats on April 26 and 27 in 1937, on May 5, 6, and 9 in 1938, and on May 1 and 2 in 1939. The material was transplanted into the field on May 26 and 27 in 1937, on June 1 in 1938, and on May 31 and June 2 in 1939. All operations were performed on the basis of blocks. Randomization within blocks prevented the danger of confounding any differences due to operations with differences due to crosses, parents, or generations.

The plots were 24 plants long in 1937 and 1938, and 12 plants long in 1939. In 1937 the plants were spaced 3½ feet apart within the plot and the plots were 3½ feet apart. This provided a spacing of 3½ by 3½ feet for plants. In both 1938 and 1939 the spacing was 4 by 4 feet. The greater spacing of 1938 and 1939 materially facilitated the taking of notes, as all notes were taken on the basis of individual plants.

The seeds were sown late in an effort to avoid any blooming of plants before transplanting into the field. In 1937 only two plants had bloomed before transplanting, in 1938 none, and in 1939 five. The material was grown under irrigation; and sufficient water was supplied during the spring, summer, and early fall to keep the plants in a vigorous growing condition.

In 1937, the F_1 generation was grown with the parents in a separate randomized-block planting because of the smaller number of F_1 plants available. All comparisons involving this generation are made through the parents in the two sets of randomized blocks. This accounts for the large standard errors of the F_1 and of the theoretical means for 1937.

One of the environmental influences should be mentioned. Severe hailstorms on July 15, 1937, and on July 13, 1938, caused quite uniform damage over the plantings in both years. To overcome possible differences due to hail injury, all plants were uniformly pruned after the hail, all remaining fruits, flowers, and flower buds being removed. In 1937 first-bloom data were obtained both before and after hail damage. Although, in 1937, 12 percent of the plants had not bloomed before the hail, the authors believe that these data taken before hail have value. Therefore, 2 days were added to the period from planting the seed to the date of the hailstorm, and this value was recorded as the number of days from the planting of the seed to first bloom for those plants that had not bloomed. This will be mentioned again in the interpretation of the results.

A clear understanding of the stages involved in the earliness-of-maturity character, as measured by number of days from seeding to first complete change of color of any fruit, is essential to a proper interpretation of the results. As previously stated, this character was divided into the following stages: Number of days from seeding to first bloom, number of days from first bloom to first fruit set, and number of days from first fruit set to first complete change of color of any fruit.

The individual plants were easily classified on the basis of these stages of development. In classifying plants for the first stage of development, the plants were recorded as having bloomed on the day that the petals opened out. For the second stage of development, the fruit was considered as having set when the ovary showed definite enlargement. The plants were examined regularly thereafter until the fruits were definitely established on the plants, to determine if sloughing occurred. Under the conditions of this experiment, sloughing of fruits rarely if ever occurred. This second stage comprised the period after flowering necessary for definite enlargement of the ovary and the period during which flowers might have been sloughed. The period from the time of blooming of any one flower until its ovary has definitely enlarged is longer for some varieties than for others. Also, in some years, all of the blossoms do not set fruit, but instead an abscission layer is formed and the blossoms drop. Hence, the stage from first bloom to first fruit set is prolonged. In establishing the third stage of development, the first complete change of color was recorded as that time when the green in the fruit had disappeared. On any specified fruit this date could be determined within a day.

The method employed in reducing the data was essentially the same as that used by Powers (*29*), with the exception of some minor changes desirable because of the nature of the experiment. Only these changes need be mentioned here. Since the primary interest lay in comparisons between averages of parents or generations, the plot instead of the plant was used as the unit for reducing the data. Then, for each parent and generation the standard errors were estimated for within crosses from the averages of plots, which were derived from single-plant data. In all tables the standard errors of the means are given. Differences having P values less than 0.05 were considered as statistically significant. The symbol B_1, which appears both in the tables and in the text, signifies that the progeny studied resulted from backcrossing the F_1 to the designated parent.

In either breeding or genetic studies the problems involved are generally simplified if the characters under investigation can be properly partitioned into their components. This is readily appreciated when one stops to consider that the number of genes differentiating any particular component of a character must be less than the number differentiating the character itself. Hence, both the breeding and genetic facts are made more intelligible by partitioning the character into its components. The earliness character, measured by the number of days from seeding to first complete change of color, with which these studies are concerned, seems to be composed of the three stages of development already specified; namely, number of days from seeding to first bloom, number of days from first bloom to first fruit set, and number of days from first fruit set to first complete change of color.

Before proceeding further, it is desirable to examine the data for the purpose of determining whether these stages of development are subcharacters of number of days from seeding to first complete change of color. If they are subcharacters, variation as to the duration of the different stages of development may be expected among the parents and generations. Likewise, different combinations of the duration of the different stages of development may be expected to occur among the variants (parents and generations). For example, all the stages of development may be of short duration in one variant, all of long duration in another variant, and the first two may be of short duration and the last of long duration in a third variant. Obviously, other combinations are possible but need not be given, as those already presented are sufficient to illustrate the point.

EXPERIMENTAL RESULTS

SUBDIVISIONS OF EARLINESS CHARACTER

JOHANNISFEUER × RED CURRANT AND DANMARK × JOHANNISFEUER

For the cross Johannisfeuer × Red Currant the data that have a bearing upon the problem as to whether the three stages of development form subcharacters of number of days from seeding to first complete change of color of any fruit are given in table 1. An examination of these data reveals that the period from seeding to first bloom is of short duration for Red Currant and the F_1 generation and of long duration for Johannisfeuer. For number of days from first bloom to first fruit set, the differences between means are not great. However, for 1939 the F_1 generation, in comparison with the parent varieties, has a definitely shorter period from first bloom to first fruit set. These same comparisons for 1937 are not conclusive because of the large standard error for the mean of the F_1 generation. The period from first fruit set to first complete change of color of any fruit is short for the F_1 generation and for Johannisfeuer. It is short for Red Currant also in 1937 but somewhat longer in 1939. The fact that the means of the F_1 generation are the smallest for the character number of days from seeding to first complete change of color of any fruit indicates that all three of the stages of development for the F_1 generation are of short duration.

For the cross Danmark × Johannisfeuer, the means, expressed in number of days (table 1), show that the period from seeding to first bloom is comparatively short for Danmark and comparatively long for Johannisfeuer. The means of the generations for this stage of development fall in between the means of the two parents, grading from one parent to the other, depending on the closeness of the genetic relationship. For the second stage of development, the F_1 generation has the smallest mean in each year and the means of the other generations approach the means of one or the other parent as the generation being considered approaches a particular parent in genetic relationship. There are no statistically significant differences between the means of the parents as regards number of days from first bloom to first fruit set. Danmark has a long period from first fruit set to first complete change of color of any fruit, whereas this same period for Johannisfeuer is short. The means of the different generations fall between the means of the two parents, becoming larger as the generations approach the Danmark parent in genetic relationship.

TABLE 1.—*Means, expressed in number of days, for different stages of development for the crosses Johannisfeuer × Red Currant in 1937 and 1939 and Danmark × Johannisfeuer in 1938–39*

JOHANNISFEUER × RED CURRANT

Parent or generation	Period from seeding to first bloom in— 1937	Period from seeding to first bloom in— 1939	Period from first bloom to first fruit set in— 1937	Period from first bloom to first fruit set in— 1939	Period from first fruit set to first complete change of color in— 1937	Period from first fruit set to first complete change of color in— 1939	Period from seeding to first complete change of color in— 1937	Period from seeding to first complete change of color in— 1939
	Days	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*
Red Currant	117.1±0.109	71.8±0.728	3.1±0.114	[illegible]0±0.1[illegible]	42.5±0.580	44.3±0.401	152.7±0.395	126.9±0.612
B₁ to Red Currant	106.9± .091	71.0± .763	[illegible].1± .014	4.4± .1 3	41.8± .308	47.1± .535	151.8± .314	123.1± .726
F₁ generation	1[illegible]7.3± .840	71.5± .3	[illegible].4± .384	3.[illegible]± .110	39.3±1.185	43.5± .472	149.9±1.111	118.4± .568
F₂ generation	108.1± .0[illegible]8	70.7± .420	3.2± .017	4.3± .142	42.0± .148	44.5± .608	153.3± .1[illegible]5	125.5± .644
B₁ to Johannisfeuer	108.4± .077	70.5± .580	3.5± .024	4.0± .12	41.4± .1[illegible]9	44.0± .498	155.2± .187	124.7± .9[illegible]0
Johannisfeuer	111.[illegible]± .271	86.4± .776	3.4± .070	4.5± .100	42.7± .238	47.2± .483	158.5± .427	136.1± .538

DANMARK × JOHANNISFEUER

Parent or generation	Period from seeding to first bloom in— 1938	Period from seeding to first bloom in— 1939	Period from first bloom to first fruit set in— 1938	Period from first bloom to first fruit set in— 1939	Period from first fruit set to first complete change of color in— 1938	Period from first fruit set to first complete change of color in— 1939	Period from seeding to first complete change of color in— 1938	Period from seeding to first complete change of color in— 1939
	Days	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*
Johannisfeuer	104.[illegible]±0.[illegible]4	[illegible].4±0.[illegible]	[illegible].0±0.045	4.[illegible]±0.100	54.7±0.[illegible]04	4[illegible].2±0.483	164.4±0.900	136.1±0.838
B₁ to Johannisfeuer	[illegible]02.7± .318	[illegible]± .8[illegible]	[illegible] .0[illegible]4	4.1± .1[illegible]	[illegible].8± .372	47.6± .[illegible]4	16[illegible].0± .463	133.3± .757
F₁ generation	[illegible]01.1± .[illegible]40	[illegible].[illegible]± .[illegible]81	1± .08[illegible]	3.4± .119	[illegible]± .4[illegible]	48.0± .516	16[illegible].6± .617	132.4± .440
F₂ generation	101.4± .2[illegible]3	[illegible]± .610	3± .0 4	4.4± .240	[illegible].7± .2[illegible]2	49.9± .412	16[illegible].4± .437	134.9± .727
B₁ to Danmark	10[illegible].6± .3[illegible]	[illegible].2± .743	4± .0 1	4.4± .22	61.6± .31[illegible]	[illegible].3± .621	167.6± .3[illegible]2	134.8± .963
Danmark	[illegible].4± .[illegible]1	7[illegible].2± .[illegible]	[illegible] .1[illegible]	4.9± .25[illegible]	[illegible].0± .4[illegible]	4[illegible].6± .[illegible]3	170.0± .4[illegible]	137.7± .632

DANMARK × RED CURRANT AND JOHANNISFEUER × BONNY BEST

The means, expressed in number of days, for different stages of development for the crosses Danmark × Red Currant and Johannisfeuer × Bonny Best are listed in table 2. As regards the Danmark × Red Currant cross, the F_1 generation has the shortest period for all three stages of development. Red Currant (tables 1 and 2) has a comparatively short period for all three stages of development, whereas Danmark (tables 1 and 2) has a comparatively short period from seeding to first bloom and a comparatively long period from first fruit set to first complete change of color of any fruit. From table 2 it can be seen that Bonny Best has a comparatively long period for all three stages of development, whereas Johannisfeuer has a long period from seeding to first bloom. The F_1 of Johannisfeuer × Bonny Best has a short period from seeding to first bloom and from first bloom to first fruit set. The period from first fruit set to first complete change of color of any fruit for the F_1 is only 2.6 days longer than the same period for Johannisfeuer.

TABLE 2.—*Means, expressed in number of days, for different stages of development for the crosses Danmark × Red Currant in 1938 and Johannisfeuer × Bonny Best in 1939*

DANMARK × RED CURRANT 1938

Parent or generation	Period from seeding to first bloom	Period from first bloom to first fruit set	Period from first fruit set to first complete change of color of any fruit	Period from seeding to first complete change of color of any fruit
	Days	*Days*	*Days*	*Days*
Red Currant	98.6±0.352	5.4±0.128	53.0±0.564	156.9±0.822
B_1 to Red Currant	97.9± .310	5.1± .056	52.0± .428	155.0± .472
F_1 generation	97.5± .528	4.8± .061	51.5± .609	153.8± .730
F_2 generation	98.5± .308	5.2± .057	53.0± .312	156.6± .180
B_1 to Danmark	98.3± .302	5.1± .055	56.4± .257	159.7± .315
Danmark	100.0± .162	5.6± .103	64.5± .435	169.8± .511

JOHANNISFEUER × BONNY BEST 1939

Parent or generation	Period from seeding to first bloom	Period from first bloom to first fruit set	Period from first fruit set to first complete change of color of any fruit	Period from seeding to first complete change of color of any fruit
Johannisfeuer	86.1±0.756	4.5±0.160	45.2±0.482	136.1±0.838
B_1 to Johannisfeuer	84.4± .613	4.7± .166	46.8± .489	136.1± .76
F_1 generation	82.9± .776	4.3± .146	47.8± .479	131.9± .736
F_2 generation	83.7± .711	4.5± .170	49.5± .558	137.5± .851
B_1 to Bonny Best	84.7± .816	4.5± .175	50.1± .659	139.2±1.006
Bonny Best	86.2± .776	5.2± .222	55.5± .783	140.8± .908

SUMMARY FOR ALL CROSSES

It is desirable at this time to summarize the evidence as to whether the designated stages of development are subcharacters of number of days from seeding to first complete change of color of any fruit. From the fact that the maximum difference between the means of any of the comparable variants was only 1.1 days for number of days from first bloom to first fruit set and from the fact that the largest mean for any variant was only 5.7 days, it may be concluded that this stage of development is of comparatively short duration for all the variants. However, statistically significant differences were shown to occur. All three stages of development were found to be of short duration for the F_1 generation of the cross Danmark × Red Currant. Red Currant was found to have short periods from seeding to first bloom and from first fruit set to complete change of color, whereas Bonny Best was found to have long periods for these same two stages of development and in addition to have the longest period from first bloom to first fruit set of any variety or generation with which it is comparable. In Johannisfeuer a long period from seeding to first bloom is combined with a short period from first fruit set to first complete change of color of any fruit; whereas in Danmark a short period from seeding to first bloom is combined with a long period from first fruit set to first complete change of color. It is apparent that variations occur in the duration of these three stages of development. Also, the findings for Johannisfeuer and Danmark prove that these variations may occur in different combinations. Hence, one may conclude that number of days from seeding to first complete change of color of any fruit is composed of the three substages indicated and that therefore the divisions made are biologically sound.

It might be well to point out that the authors do not contend that the character studied might not be partitioned further to advantage. For example, it might be advantageous to divide the stage number of days from seeding to first bloom into number of days required for germination and number of days from germination to first bloom. This was not done because of the difficulties involved in obtaining individual plant data on germination.

EFFECT OF ENVIRONMENT ON STAGES OF DEVELOPMENT

Having found that the stages of development as designated are biologically sound, it is desirable to examine the data for the purpose of determining the influence that the environment has upon an interpretation of the data. Information concerning the influence of environment may be obtained by comparing the number of days from seeding to first bloom before and after the hailstorm in 1937 and by comparing the differences between years for the crosses Johannisfeuer×Red Currant and Danmark×Johannisfeuer.

When interpreting the data for mean number of days from seeding to first bloom before and after hail for each parent and generation (table 3), it should be kept in mind that 12 percent of the plants had not bloomed before the hailstorm occurred and that, in calculating the means and standard errors, all of these plants were considered as having bloomed on what would have been the next date for taking bloom data. Bloom data were taken three times a week. It is apparent that the means of all the parents and generations would not be affected to the same degree by such a procedure but that the means of the later maturing parent and generations would be somewhat smaller than they should be in comparison with the means of the earlier maturing parent and generations. To take a specific example, the difference before hail of 6.9 days between the mean of Johannisfeuer and the mean of Red Currant is in all probability somewhat less than it would have been if hail had not occurred. With these facts in mind, the data listed in table 3 may be interpreted more logically. It can be seen that the rank as to magnitude before hail and after hail is the same for the parents and generations with one exception; that is, in the comparison of the mean of Red Currant with the mean of the B_1 to Red Currant. Since in no instance are the differences between the means of the Red Currant parent and the means of the progeny obtained by backcrossing the F_1 generation to Red Currant statistically significant, the exception noted cannot be attributed logically to an interaction between parents or generations and the environment but can be accounted for by chance fluctuation. The difference between the mean of Red Currant and the mean of Johannisfeuer before hail (6.9) is too much greater than the difference between means of the same varieties after hail (4.8) to be accounted for by the probable errors of random sampling. The same conclusion holds for other similar comparisons involving generations or generations and parents. Then the only apparent effect of hail damage has been to decrease the magnitude of the differences between parents, of differences between generations, and of differences between parents and generations. Such being the case, the interpretation of the data as to the biological justification of the division number of days from seeding to first bloom has not been affected by environmental conditions resulting from hail damage.

TABLE 3. *Mean number of days from seeding to first bloom before and after hail for each parent and generation of the cross Johannisfeuer × Red Currant in 1937*

Parent or generation	Period from seeding to first bloom	
	Before hail	After hail
	Days	*Days*
Red Currant	84.2±0.277	107.1±0.109
B_1 to Red Currant	84.7±.313	106.9±.091
F_1 generation	85.4±.358	107.3±.510
F_2 generation	87.2±.129	108.1±.058
B_1 to Johannisfeuer	88.6±.114	108.1±.077
Johannisfeuer	91.1±.223	[illegible]

In regard to the effect of environment, as represented by years, upon an interpretation of the data, the means for the cross Johannisfeuer × Red Currant for the years 1937 and 1939 (table 1) show that the rank of the stage number of days from seeding to first bloom is the same (within the limits of sampling error) for both years. Consequently, the same interpretation as to the biological soundness of this stage as a division of the character number of days from seeding to first complete change of color in any fruit would have been placed upon the data if the experiment had been conducted for only 1 of the 2 years.

As to the second stage, in 1937 the mean for the Red Currant variety was significantly smaller than the mean for Johannisfeuer, whereas in 1939 the reverse was true. The data pertaining to the second stage show that in 1938 the mean for Johannisfeuer was 5.6±0.093 (table 1) and for Red Currant 5.4±0.128 days (table 2). These means are not significantly different if odds as great as 19 : 1 against the difference noted being due to the probable errors of random sampling are accepted as a criterion of significance. The data do not agree for any of the 3 years. It would seem that environmental conditions as represented by years interact with parents and generations in such a manner that the ranking of the means of the parents is not the same for the different years. The environment must be taken into account in studying this stage of development. However, the conclusions as to the soundness of considering this stage of development a subdivision of the character under investigation is shown by the data for both 1937 and 1939.

Finally, in regard to the third stage of development for the cross *Johannisfeuer*×Red Currant (table 1) a detailed discussion of the data is not necessary, as it is apparent that in general the conclusions are the same as those drawn for the stage number of days from first bloom to first fruit set.

The data for the cross Danmark×Johannisfeuer in 1938–39 (table 1) show that for all three stages the rank of the means as to magnitude is the same for both years, with the possible exception of comparisons involving the B_1 to Johannisfeuer, the F_2 generation, and the B_1 to Danmark. These possible exceptions are for number of days from first bloom to first fruit set. Even these exceptions cannot be attributed to interactions between the environment and generations, as the differences between generations can be accounted for by the probable errors of random sampling (*39, p. 54, table 10*). In these cases, also, the interpretation placed upon the data as to the biological soundness of the stages of development as subdivisions of the character number of days from seeding to first complete change of color of any fruit is the same for both 1938 and 1939.

In summarizing the data for both crosses, it can be said that the environmental conditions as represented by years do not alter the interpretation placed upon the data as to whether the designated stages of development are justified biologically. This does not mean that environmental conditions do not have an influence upon the duration of the different stages of development. On the contrary, the data in table 1 lead one to conclude that environment has a decided effect on all stages of development.

HETEROSIS AND DOMINANCE

Accepting Jones' well-known theory (*16*) of heterosis as being the most satisfactory for explaining the known facts regarding this phenomenon, it seems logical to discuss heterosis and dominance under the same heading. This does not mean that the authors consider that, from the standpoint of physiological genetics, heterosis and dominance are fundamentally different. In fact Jones' hypothesis (*16*) for the explanation of heterosis assumes at least the existence of partial dominance of the factors affecting the character under consideration. In this study the term "heterosis" is applied to those cases in which the mean period of development of the F_1 is less than the mean of either parent. In other words, the ability to complete the different stages of development in a shorter period of time is taken to be an expression of heterosis. The authors would like to point out that they do not mean to imply that a different criterion of heterosis would not be desirable for other studies.

Complete dominance is attributed in those instances in which the phenotype of the F_1 is that of one or the other parent. At the other extreme, when the phenotype of the F_1 generation is the arithmetic mean of the phenotypes of the two parents, dominance is considered to be nonexistent. Finally, when the mean of the F_1 generation falls between the means of the two parents but not exactly midway (arithmetically), the parental phenotype most closely resembled is considered to be partially dominant. Hence, like Mendel (*25*), Naudin (*26*), and other earlier workers, the authors have considered dominance to be a phenomenon of the phenotype. Also, since the means of those F_1's exhibiting heterosis fit neither the arithmetic nor the geometric mean calculated from the means of the two parents, it seems desirable at this time to use the earlier method of determining dominance: namely, that of ascertaining which parental phenotype the F_1 more nearly approaches.

JOHANNISFEUER × RED CURRANT

The data for the cross Johannisfeuer × Red Currant (table 1) show that significant differences between the means of Red Currant, the B_1 to Red Currant, and the F_1 generation do not exist for number of days from seeding to first bloom for either 1937 or 1939. For all practical purposes the phenotype of the Red Currant parent is completely dominant. Also, it can be seen that in 1939 a significant difference did not exist between the mean of the F_2 and the mean of the B_1 to Johannisfeuer for the same subcharacter. The difference for 1937, although statistically significant, is small. The means of the Johannisfeuer parent for 1937 and 1939 are significantly larger than the means for any other variant.

Considering the data for number of days from first bloom to first fruit set, it can be seen that for 1937 the standard error of the mean of the F_1 generation is too large to allow the drawing of conclusions regarding either heterosis or dominance. For 1939 it is clearly evident that heterosis does exist, as the F_1 mean is significantly smaller than the mean of either parent, and furthermore in no case is the mean of any of the generations as large as the mean of the parent to which the generation is most closely related. However, the differences noted in this latter comparison are not always statistically significant.

The data for the third stage likewise show that the mean of the F_1 is significantly smaller than the mean of either parent for each year of this test. This shows that heterosis does occur for this stage of development. Also, each of the generation means, with the exception of that of the B_1 to Red Currant in 1939, is smaller than the mean for either parent. Again, the differences noted in this latter comparison are not always statistically significant.

For the earliness character, measured by number of days from seeding to first complete change of color, the means of the F_1 generation are significantly smaller than the mean for either parent for both 1937 and 1939, and furthermore each of the generation means for 1939 is smaller than the mean for either parent, although the differences are not always statistically significant.

DANMARK × JOHANNISFEUER

The data for the cross Danmark × Johannisfeuer (table 1) show that the mean of the F_1 generation falls between the means of the two parents for number of days from seeding to first bloom but is closer to the mean of the Danmark parent. Hence, earliness of bloom is partially dominant. The means of all the generations fall between the means of the two parents. Considering both 1938 and 1939, the differences between generation and parental means are statistically significant.

Regarding number of days from first bloom to first fruit set, the mean of the F_1 generation for each year is significantly smaller than the mean of either parent. Again, for both years the means of all generations are smaller than the mean for either parent. However, the differences are not always statistically significant.

For number of days from first fruit set to first complete change of color, the mean of the F_1 generation lies between the means of the two parents but closer to the mean of the parent having a short period for this stage of development, and each of the generation means lies between those of the two parents. By the criterion used, no heterosis exists; but as was found for the first stage, short duration of the period involved is partially dominant.

From an examination of the data for number of days from seeding to first complete change of color, which is the summation of the previous stages, it is found that in 1938 the means of Johannisfeuer, the B_1 to Johannisfeuer, and the F_1 are not significantly different, and that each of the generation means falls between the means of the two parents. This would indicate complete dominance of short duration of the period from seeding to first complete change of color of any fruit. For 1939 this same character showed heterosis, the period from seeding to first complete change of color of any fruit being significantly shorter for the F_1 than for either parent. The same was true for all other generations as well, but the differences between the generation means and the parental means were not always statistically significant.

DANMARK × RED CURRANT AND JOHANNISFEUER × BONNY BEST

The data concerning heterosis and dominance for the crosses Danmark × Red Currant and Johannisfeuer × Bonny Best are given in table 2. In the case of the cross Danmark × Red Currant, all subcharacters and the character number of days from seeding to first complete change of color of any fruit have F_1 means significantly smaller than the comparable means of either parent, with the exception of the second subcharacter, in which case the difference divided by the standard error gives a P value somewhat larger than 0.05. Accordingly, with the possible exception of number of days from first fruit set to first complete change of color of any fruit, all the stages of development and the combination exhibit heterosis. In the cross Johannisfeuer × Bonny Best, heterosis is exhibited for the first and second stages. However, in the latter the difference between the F_1 and the Johannisfeuer parent is not statistically significant. For the first subcharacter, each of the generation means is less than the mean of either parent. For number of days from first fruit set to first complete change of color of any fruit, the mean of the F_1 generation lies between the means of the two parents but is closer to the mean of Johannisfeuer. Hence, the shorter period of this stage of development is partially dominant. The F_1 mean for number of days from seeding to first complete change of color of any fruit is smaller than that of either parent. However, the difference between the F_1 mean and the mean of the Johannisfeuer parent is not statistically significant.

SUMMARY FOR ALL CROSSES

Heterosis occurred in the Danmark × Red Currant and the Johannisfeuer × Bonny Best crosses, as is indicated by smaller number of days from seeding to first bloom. On the basis of number of days from first bloom to first fruit set, heterosis occurred in all crosses in all years, with the possible exceptions of Johannisfeuer × Red Currant in

1937 and Johannisfeuer × Bonny Best in 1939. In the Johannisfeuer × Red Currant cross, because of the large standard error of the F_1 generation, no definite conclusions could be drawn. Heterosis for the third developmental stage was exhibited by the cross Johannisfeuer × Red Currant. For this same stage, the mean of the F_1 generation in the cross Danmark × Red Currant was smaller than the mean of either parent but the difference between the mean of this generation and the mean of the Red Currant parent was not statistically significant. The character number of days from seeding to first complete change of color of any fruit exhibited heterosis in the crosses Johannisfeuer × Red Currant, Danmark × Johannisfeuer (in 1939 but not in 1938), and Danmark × Red Currant. For this character, the mean of the F_1 in the cross Johannisfeuer × Bonny Best was less than the mean of either parent but the difference involved in the comparison with Johannisfeuer was not statistically significant. In conclusion, it can be said that both heterosis and dominance are dependent upon the cross and upon environment as represented by years.

EFFICIENCY OF THEORETICAL MEANS IN DETERMINING WHETHER THE EFFECTS OF THE GENES ARE ARITHMETICALLY OR GEOMETRICALLY CUMULATIVE

MacArthur and Butler (*24*), Charles and Smith (*2*), and Powers (*29*) have used the fit between obtained and theoretical means as a method of determining whether the data could be explained more logically on the assumption that the effects of the genes are arithmetically cumulative or on the assumption that they are geometrically cumulative. Much of the theory basic to the validity of this method is given by Charles and Smith (*2*) in developing the formulas for obtaining the theoretical means. This theory need not be repeated here. The necessity of considering dominance of the characters with which they worked was pointed out by Lindstrom (*21*) and Powers (*29*). Table 4 shows the formulas for estimating the theoretical means that take dominance and heterosis into consideration.

The formulas for the means of the F_2 generation were developed by Wright, as cited by Powers (*29*). In applying these formulas, in other words, in using this method, one should ascertain the efficiency of the method. If it is efficient, the difference between the two comparable theoretical means of generation or backcross must be statistically significant or, expressed in another way, the contrasted formulas must be discriminatory. If they are not discriminatory, the difficulty may be inherent in the data or it may be inherent in the method as applied to such data.

TABLE 4. *Formulas for estimating arithmetic and geometric means*

Generation or backcross	Arithmetic mean	Geometric mean
F_2 generation	$\frac{\bar{P}_1+2\bar{F}_1+\bar{P}_2}{4}$	Antilogarithm of $\frac{\log \bar{P}_1+2\log \bar{F}_1+\log \bar{P}_2}{4}$
B_1 to P_1	$\frac{\bar{F}_1+\bar{P}_1}{2}$	Antilogarithm of $\frac{\log \bar{F}_1+\log \bar{P}_1}{2}$
B_1 to P_2	$\frac{\bar{F}_1+\bar{P}_2}{2}$	Antilogarithm of $\frac{\log \bar{F}_1+\log \bar{P}_2}{2}$

The data may be tested by two criteria: (1) If the differences between parents are of sufficient magnitude to be of economic importance, methods should be developed by which problems involving these differences can be studied; (2) if the differences are of sufficient magnitude to be established as statistically significant, methods should be developed by which problems involving these differences can be studied because such differences are important to theoretical genetics if not at the moment of importance to applied genetics. From tables 1 and 2 it can be seen that, with the possible exception of the stage from first bloom to first fruit set, economically important differences exist for all stages of development and for the earliness character measured by number of days from seeding to first complete change of color of any fruit. Hence any difficulties noted would not be inherent in the data.

TABLE 5.—*Obtained and theoretical means for different stages of development of the cross Johannisfeuer × Red Currant, expressed in number of days*

Year and parent or generation	Period from seeding to first bloom: Obtained	Period from seeding to first bloom: Theoretical, Arithmetic	Period from seeding to first bloom: Theoretical, Geometric	Period from first bloom to first fruit set: Obtained	Period from first bloom to first fruit set: Theoretical, Arithmetic	Period from first bloom to first fruit set: Theoretical, Geometric	Period from first fruit set to first complete change of color: Obtained	Period from first fruit set to first complete change of color: Theoretical, Arithmetic	Period from first fruit set to first complete change of color: Theoretical, Geometric	Period from seeding to first complete change of color: Obtained	Period from seeding to first complete change of color: Theoretical, Arithmetic	Period from seeding to first complete change of color: Theoretical, Geometric
1937	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*
B_1 to Red Currant	106.9±0.091	107.2±0.275	107.2	3.1±0.019	3.2±0.195	3.2	41.8±0.308	40.9±0.766	40.9	151.8±0.319	151.3±0.590	151.3
F generation	108.1±.058	108.4±.280	108.1	3.2±.017	3.4±.195	3.4	42.0±.148	41.0±.751	40.9	153.3±.155	152.5±.571	152.7
B_1 to Johannisfeuer	108.4±.077	109.6±.302	109.6	3.3±.024	3.6±.198	3.6	41.4±.159	41.0±.752	41.0	153.2±.187	154.2±.595	154.1
1939												
B_1 to Red Currant	71.0±.763	71.7±.457	71.7	4.9±.153	4.5±.095	4.4	47.1±.535	46.4±.341	46.3	123.1±.726	122.5±.417	122.4
F_2 generation	76.7±.920	75.3±.781	75.0	4.3±.112	4.3±.078	4.3	44.5±.608	45.4±.293	45.3	125.5±.611	125.0±.385	124.8
B_1 to Johannisfeuer	76.5±.586	79.0±.476	78.6	4.0±.125	4.2±.097	4.2	44.0±.498	44.4±.338	44.3	124.7±.970	127.7±.506	127.2

To determine the efficiency of the method as applied to these data, the obtained and theoretical means for different stages of development in the cross Johannisfeuer×Red Currant are tabulated in table 5. In no case is there a statistically significant difference between the obtained mean and the corresponding theoretical mean. The comparable data in the latter half of table 1 and in table 2 for the other crosses were calculated; but, since the conclusions drawn were the same as those drawn for the data presented in table 5, it is not necessary to present the data in detail. It is apparent that, as regards this study, this method is not sufficiently sensitive to be of any particular value as a means of studying the nature of the interactions of the genes. The agreement between the obtained means and the theoretical means was good with the possible exception of the B_1 to Johannisfeuer in 1939 for number of days from seeding to first bloom (table 5). Also, the agreement between the obtained and the theoretical means was good for the other crosses. Because of the inefficiency of the method employed, conclusions drawn from such facts would be of little, if any, value.

DISCUSSION AND CONCLUSIONS

From the experimental data it was found that the earliness-of-maturity character, as measured by number of days from seeding to first complete change of color of any fruit, is composed of at least three stages of development: Number of days from seeding to first bloom, number of days from first bloom to first fruit set, and number of days from first fruit set to first complete change of color of any fruit. This fact has considerable importance in breeding early varieties of tomatoes. The cross Danmark × Johannisfeuer may be used to illustrate this point. It will be remembered that Danmark has a short period of development from planting of the seed to first bloom but a long period of development from first fruit set to first complete change of color of any fruit, whereas Johannisfeuer has a long period of development from planting of the seed to first bloom but a short period of development from first fruit set to first complete change of color of any fruit. Now, since the F_1 generations of the crosses Danmark × Red Currant and Johannisfeuer × Red Currant have short periods for both of these stages of development, it would seem that a variety could be produced recombining the genes for short duration of the stage of development from seeding to first bloom, characteristic of Danmark, with the genes for short duration of the stage of development from first fruit set to first complete change of color of any fruit, characteristic of Johannisfeuer. Adverse linkage relationships would complicate the problem. Also, the nature of the interactions of the recombined genes would determine whether a strain could be developed that possessed a short period for both of these stages of development. At the present time neither the linkage relationships nor the nature of the interactions of the genes are known.

The importance of heterosis to a breeding program was recognized by early workers: Kölreuter (*19*), Knight (*18*), Sageret (*31*), Gärtner (*9*), Focke (*8*), Shull (*33*, *34*, *35*, *36*), East (*4*, *5*, *6*), East and Hayes (*7*), Jones (*17*), and many others. The possibility of utilizing heterosis in commercial production of tomatoes has been pointed out by Wellington (*40*), Hayes and Jones (*14*), and others. Jones (*17*), reporting on earliness in tomatoes, a large part of the data having been collected by H. K. Hayes, states that in one cross the F_1 generation was fully as early as the early parent. Luckwill (*22*) found that the time of flowering in tomato hybrids was intermediate between that of their parents, except in intraspecific crosses that differed in a major growth factor (*d* or *br*), when dominance of early flowering was shown. In regard to utilizing heterosis in tomato production, the possibility of making profitable the use of F_1 seed to produce the commercial crop of tomatoes should be considered. The authors realize that this problem cannot be completely solved at this time. However, the data on earliness of maturity as reported in this paper have some bearing upon the solution.

The data having a bearing upon heterosis as expressed by shortening of the period from seeding to first complete change of color of any fruit are listed in table 6. Upon examination of these data, it is found that only 2 of the 12 differences are not statistically significant. The F_1 generations are not significantly earlier than Johannisfeuer in either the cross Danmark × Johannisfeuer in 1938 or the cross Johannisfeuer × Bonny Best. Special attention should be given to the crosses Johannisfeuer × Red Currant and Danmark × Red Currant. For the cross Johannisfeuer × Red Currant, the period from seeding to first complete change of color in 1937 was 2.8 days shorter for the F_1 than for the Red Currant parent and 8.6 days shorter for the F_1 than for the Johannisfeuer parent; in 1939 this same period was 7.1 days shorter for the F_1 than for the Red Currant parent and 17.2 days shorter than for the Johannisfeuer parent. For the cross Danmark × Red Currant, the period from seeding to first complete change of color of any fruit was 16 days shorter for the F_1 than for Danmark and 3.1 days shorter than for Red Currant. It may be concluded that heterosis is quite pronounced in the cross Johannisfeuer × Red Currant and the cross Danmark × Red Currant.

TABLE 6.—*The differences obtained by subtracting the parental means from their respective F_1-generation means for number of days from seeding to first complete change of color of any fruit*

Cross and parent	Differences[1] between means (cross minus parent)		
	1937	1938	1939
	Days	*Days*	*Days*
Johannisfeuer × Red Currant			
Johannisfeuer	8.6±1.190		17.2±1.012
Red Currant	−2.8±1.179		−7.1±.835
Danmark × Johannisfeuer			
Danmark		−1.4±0.787	5.3±0.770
Johannisfeuer		*.7±1.091	−3.7±.946
Danmark × Red Currant			
Danmark		−16.0±.910	
Red Currant		−3.1±1.090	
Johannisfeuer × Bonny Best			
Johannisfeuer			
Bonny Best			11.9±1.160

[1] P 0.01 for all differences not marked with an asterisk (*), but 0.05 for all values marked with an asterisk.

In evaluating the shortened period from seeding to first complete change of color of any fruit exhibited by the F_1 generation, it is well to consider some of the medium- to large-fruited varieties available for commercial production. Babb and Kraus (*1*) have found Johannisfeuer to be the earliest in maturity of the medium- to large-fruited varieties and Danmark to be the earliest variety that produces fruits of sufficient size, smoothness, and quality to be classified as a fairly acceptable commercial variety. Bonny Best is classified as a second early by Boswell[3] and is one of the most widely grown varieties. In 1939 the F_1 of Johannisfeuer × Red Currant required an average of 17.2 fewer days from seeding to first complete change of color of any fruit than did Johannisfeuer, and 27.9 fewer days than did Bonny Best. In 1938 the F_1 generation of Danmark × Red Currant required an average of 16.0 fewer days from seeding to first complete change of color of any fruit than did Danmark. These figures amply demonstrate the advantage in earliness that these F_1 generations have over the earliest maturing commercial varieties. If this advantage of the F_1 generations can be transferred to the F_1 generations of commercial varieties, such a new production will be of decided benefit to those sections having a short growing season; for example, the high irrigated valleys of the Rocky Mountains and the northern parts of the United States. It seems possible that the use of F_1 seed may have a place in commercial tomato production, the F_1 being grown until the plant breeder, if possible, recombines the desirable characteristics of both varieties into a single variety as early as the F_1.

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[3] Personal correspondence.

The great importance to the plant breeder of information concerning the nature of the interactions of the genes affecting earliness of maturity may be deduced from the foregoing discussion. Therefore, methods of studying the nature of the interactions of genes differentiating quantitative characters merit careful consideration.

Three methods that have been used to study the nature of the interactions of the genes differentiating quantitative characters will be discussed. In some studies the genotypes can be identified directly (*13*, *27*, *41*), and thus segregates of different genotypes can be accurately classified. Where such a procedure can be followed, this is probably, in general, the most efficient method of studying the nature of the interactions of the genes. However, in the majority of cases, the different genotypes dependent upon segregation of the genes differentiating the quantitative character cannot be determined. Then the nature of the interactions of the genes may be studied by means of markers (*3*, *28*, *29*, *30*, *37*) and possibly by comparing the averages of different generations with a predicted average calculated from parental and F_1 means. The calculation of these means is based on certain theoretical considerations (*2*, *12*, *24*, *29*).

All three of these methods have certain advantages and if applicable should furnish information concerning the nature of the interactions of the genes as measured by end products. An advantage of the first method is that genes having major effects are being studied directly through the effects they produce. However, it should be kept in mind that other genes on the same chromosome that differentiate the same character would have an effect also. An advantage of the second method is the ease with which the classification of the segregating generations can be made. By a proper design of the experiment the effects of sections of the chromosome can be measured and the nature of the interactions of the genes studied (*30*). The third method entirely dispenses with the classification within generations. As a consequence, both the work of taking the data and the work of reducing it are greatly diminished, making it possible to broaden the scope of the studies by including more crosses. Certain other facts should be kept in mind regarding this method. Lindstrom (*21*), Powers (*29*), and Lyon (*23*) have shown that dominance must be taken into account also. This may be summed up by saying that the measurements of the F_1 must be used in calculating the theoretical means. Moreover, it should be kept in mind that the generations are treated as the units and hence it is not the nature of the interactions of particular gene pairs that is measured but probably the nature of the interactions of a large number of gene pairs whose individual effects have not been determined. In other words, this type of study deals with the genes in mass. It is apparent that the information obtained by the use of one of these methods would supplement that obtained by the use of another. Hence, providing they are efficient, all would have their place in studying the nature of the interactions of the genes as measured by end products. It will be remembered that the method of comparing obtained parental and generation means with theoretical means was not sufficiently discriminative to be of any value in determining the nature of the interactions of the genes differentiating the stages of development under consideration in this study.

SUMMARY

The subdivision of the period from seeding to first complete change of color of any fruit into the following stages of development was found to be biologically sound for certain crosses involving varieties of *Lycopersicon esculentum* and *L. pimpinellifolium:* (1) Number of days from seeding to first bloom; (2) number of days from first bloom to first fruit set; (3) number of days from first fruit set to first complete change of color of any fruit.

In each stage of development heterosis was exhibited in the case of some one or other of the crosses.

Heterosis was found to be dependent upon both the genotype (cross) and the environment.

The method involving comparison of the obtained mean of a given generation with predicted arithmetic and geometric means calculated from obtained parental and F_1 means was of little value, as regards these data, in determining whether the nature of the interactions of the genes was such that the effects were arithmetically cumulative or whether it was such that the effects were geometrically cumulative

The possible importance of the findings in a plant-breeding program is discussed.

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INHERITANCE OF QUANTITATIVE CHARACTERS IN CROSSES INVOLVING TWO SPECIES OF LYCOPERSICON [1]

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INTRODUCTION

Studies on the inheritance of quantitative characters in crosses within the genus *Lycopersicon*, like all quantitative inheritance studies, are concerned with multiple factors, heterosis, dominance, and the nature of the interactions of the genes differentiating the quantitative characters. The purpose of the research reported herein was to obtain more information concerning the latter three phases of the problem and to apply this information in shaping a tomato-breeding program. The work was done at the Horticultural Field Station, Cheyenne, Wyo.

Before proceeding further, the writer wishes to point out that, even though from the standpoint of physiological genetics heterosis and dominance are probably fundamentally the same, it is convenient in these studies to use both terms. It is important, however, that the use of these terms be clearly understood. Those cases are considered as exhibiting heterosis in which the phenotypic expression of a character in the F_1 generation is either greater than or less than the magnitude of such expression in either parent. Complete dominance is applied to those cases in which the phenotypic expression in the F_1 generation is that of one or the other parent, provided there is a significant difference between the two parents as regards the character under consideration. The term "partial dominance" is used when the phenotypic expression in the F_1 generation lies between that of the parents, but is anything other than exactly intermediate (arithmetically). Finally, neither heterosis nor dominance is considered as existing when the expression in the F_1 generation is exactly intermediate (arithmetically) between the magnitudes for the two parents.

PREVIOUS RESEARCH

Without attempting to review all of the previous investigations dealing with heterosis and dominance and with the nature of the interactions of the genes as measured by end products, it is desired to recall the fundamental principles established by earlier work, as well as some of the theories dealing with the genetic explanation of these phenomena.

HETEROSIS AND DOMINANCE

Hybrid vigor has received the attention of plant students for more than a century and a half. Kölreuter (*29*) [2] reported cases of hybrid

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[2] Italic numbers in parentheses refer to Literature Cited, p. 172

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vigor as early as 1766. That hybrid vigor is of general occurrence in those F_1 generations that come from parents of genetic diversity was established early by Knight (*28*), Sageret (*41*), Gärtner (*13*), Darwin (*4*), and Focke (*12*). Inbreeding followed by hybridization, as a method of utilizing heterosis in corn improvement, was advocated by Shull (*42, 43, 44*), East (*6, 7, 8*), and East and Hayes (*11*). Their work was the basis for the development of the modern methods employed in breeding those crops in which the utilization of heterosis is a primary consideration.

The first genetic explanation of hybrid vigor was given by Keeble and Pellew (*25*) in 1911. They crossed two varieties of peas of medium size and obtained F_1 plants larger than either parent. One of the parents used in the cross had few but long internodes, whereas the other parent had numerous, short, thick internodes. The F_1 hybrid had both numerous and long internodes and therefore was considerably taller than either parent. Keeble and Pellew (*25*) found that each of these characters was differentiated by one factor pair and that the larger size of the F_1 generation was due to the fact that the effects of these two gene pairs were cumulative.

That there is an association between number of dominant factors and hybrid vigor was postulated by Bruce (*2*). Jones (*23*) followed up this supposition and developed what is probably the most generally accepted explanation of heterosis. This hypothesis is, briefly, as follows: (1) A large number of genes are responsible for the differentiation of most of the quantitative characters; and (2) those genes favorable to the production of a quantitative character are at least partially dominant. These two assumptions are sufficient to account for hybrid vigor, but may not explain why corn breeders have not been able to obtain selfed lines of corn as vigorous as the F_1 generation between two inbred lines that combine well. Jones (*23*) pointed out that, since maize has only 10 pairs of chromosomes, if a large number of gene pairs are involved some of these genes would have to be linked provided the chromosome theory of heredity is correct. Consequently, the probability would be extremely small of recombining into a single inbred line all of the genes favorable to growth of two inbred lines. Most plant breeders seeking to take advantage of heterosis have accepted this theory and have used it as a basis for their breeding programs. As is well known, corn breeders have been particularly successful in using breeding methods based on Jones' (*23*) explanation of heterosis (*18, 27*).

In 1936 East (*10*) advanced a somewhat different theory in explanation of heterosis. He divided genes into two classes, defective and nondefective, and stated that heterosis is not concerned with the defective genes. With this conception in mind, East (*10*) made the following statement: "The cumulative action of the nondefective allelomorphs of a given gene approaches the strictly additive as they diverge from each other in function." With this classification of genes in mind, East (*10*) developed the following theory. If numerous nondefective allelomorphs are common in any given species, heterosis can be accounted for as follows: The effect of $A_1 A_1$ is not $2 A_1$, but $2 A_1 - \alpha$, where the value of α approaches the value of A_1. But if $A_1 \ldots A_4$ are a series of nondefective allelomorphs of A_1, wth their functions departing from those of A_1 on an ascending scale, then the resultant effects may be visualized as $A_1 A_2$ equaling $A_1 + A_2 - \beta$, $A_1 A_3$ equaling $A_1 + A_3 - \gamma$, and $A_1 A_4$ equaling $A_1 + A_4 - \delta$, where $\alpha > \beta > \gamma > \delta$.

At this time it is desirable to compare the two theories. Both assume that a large number of factors are involved in differentiating the quantitative characters. Jones' (*23*) theory assumes at least partial dominance of these numerous favorable growth genes, whereas East's (*10*) theory assumes divergence in function between members of any one allelomorphic series. To explain why corn breeders have not been able to select inbred lines as vigorous as the more productive F_1 generation hybrids, Jones (*23*) points out that if the genes bringing about heterosis are very numerous in a species, such as *Zea mays*, with only 10 pairs of chromosomes, some of the genes favorable to growth would of necessity be linked with genes not so favorable to growth. Consequently, it would be extremely difficult to obtain inbred lines as vigorous as the F_1 generation. According to East's (*10*) theory, if only divergence in function between members of pairs of allels were operating to bring about the expression of hybrid vigor, the production of inbred lines as vigorous as the F_1 generation, originating from genetically diverse parents, would be impossible.

The evidence is not very extensive in support of the contention that divergence in function occurs between members of allels. Sinnott and Dunn (*47*) cite a case reported by Dunn, in which each of two recessive genes (t^0 and t^1) in the mouse is lethal when homozygous (t^0t^0 or t^1t^1), but the heterozygote (t^0t^1) is viable and normal in character. They point out that the action of these two probably allelic genes is complementary and hence the results can be interpreted on the basis of divergence in function of the members of the allelic pair of genes. Luckwill (*32*) believes that the results he obtained from studies dealing with heterosis can be most logically explained by East's (*10*) theory. It is clear that the partial dominance theory for the explanation of heterosis and the theory involving the divergence in function of members within allels are not mutually exclusive, since the phenomena differentiating these two theories may be operating in the same cross. That such may be the case seems probable when one considers the part that duplication of parts of chromosomes, aneuploidy, and polyploidy accompanied by point mutation must have played in the origin of divergent plant forms. Hence, both of these theories merit careful study.

Evidence sufficient to differentiate the partial-dominance theory and the theory based on divergence in function between members of allels will be difficult to obtain. However, evidence concerning the relative importance of these two phenomena will not be so difficult to obtain. For example, if it were possible to establish selfed lines as vigorous as the F_1 generation, in those cases in which the F_1 generation was materially more vigorous than the inbred lines from which it originated, then the divergence in function of members of allels, at least, could not have played a predominant part in bringing about heterosis; since, according to this theory, heterosis is dependent upon heterozygosity. The experimental design for such a test should include at least the F_1 generation, the original inbred lines from which it sprang, and the selected inbred line or lines. As far as the author can ascertain, no case involving such a critical test in which the selected inbred lines have equaled the F_1 generation in vigor has been reported for either naturally self-fertilized or naturally cross-fertilized plants.

Richey and Sprague (*40*) have done work that has a bearing upon this problem. They attempted to increase productivity of inbred lines by convergent improvement (*39*). The process involved backcrossing the F_1 generation to each of the original inbred lines entering the cross to produce the F_1 generation, and then selecting to carry over as many of the favorable factors from the nonrecurring parent as possible. These selected plants were again crossed to the recurring parent. Hence, two independent backcrossing programs were carried along simultaneously, one to one parent and another to the other. The criterion as to the number of times that backcrossing and selection should be practiced was whether the yield of the progeny obtained by crossing the selected backcrossed plants with the nonrecurring parent equaled or exceeded the yield of the F_1 generation plants obtained by crossing the original inbred lines. Richey and Sprague (*40*) found three or four generations of back-pollinating to be sufficient for the selection of some lines whose behavior in such crosses would be equal or superior to the recurring parent. Moreover, they stated that experience also indicated that only two or three generations of selfing after back-pollinating would be required to fix the recovered lines sufficiently for a second cycle of convergent improvement.

With the immediately foregoing methods and results in mind, it is easier to follow Richey and Sprague's (*40*) discussion as to the bearing of their data on the theories for the explanation of heterosis. In 1931, when they wrote their paper, East's theory had not been advanced. They examined their data to determine whether heterosis could most logically be accounted for by the physiological-stimulation hypothesis or by the hypothesis based on at least partial dominance of genes favorable to growth. However, their remarks concerning the stimulation hypothesis could just as well apply to East's theory of divergence in function of members of allels, since according to both theories heterosis is dependent upon heterozygosity. Richey and Sprague's (*40*) data pertaining to the problem under consideration were divided into two separate categories.

In the first category were given the yields of the F_2 generations, the yields of the F_1 corn crosses back-pollinated to one parent for different numbers of generations and then crossed with the nonrecurring parent, and the yields of the F_1 generations obtained by crossing the original inbred lines. Selection to retain genes from the nonrecurring parent was practiced during the back-pollinating program. Richey and Sprague (*40*) compared the obtained yields with the theoretical yields calculated on the assumption that the effects of the genes are arithmetically cumulative. That this assumption probably was correct will be shown later. They pointed out that for the first backcross generation selection could not be practiced, but that for the remaining backcross generations selection for genes from the nonrecurring parent would make these selected lines more like the nonrecurring parent. Hence, according to the theory of divergence in function between members of allels, the yields of the crosses between these selected lines and the nonrecurring parent should be lower than the theoretical yields. As a matter of fact, Richey and Sprague (*40*) found the reverse to be true, as would be expected on the basis of the partial-dominance theory for the explanation of heterosis.

In the second category were discussed the yields of F_1 generations resulting from crossing selected inbred lines and the yields of the F_1 generations resulting from crossing the original inbred lines. In any one cross the two selected inbred lines resulted from a program of backcrossing to different parents. According to the theory of divergence in function between members of allels, the F_1 generations resulting from crossing selected lines should produce lower yields than the F_1 generations resulting from crossing the original inbred lines. Richey and Sprague (*40*) obtained quite the reverse result. The F_1 crosses $(3 \times 2^4) \times (2 \times 3^4)$ and $(3 \times 2^5) \times (2 \times 3^5)$ yielded more than the actual parental F_1 cross 2×3. Again, the results are those expected on the basis of the partial-dominance theory for the explanation of heterosis. However, the results do not exclude the possibility of divergence in function of members of allels accounting for some of the heterosis exhibited by these F_1 generations; but the results do furnish rather conclusive evidence that such a phenomenon cannot be the sole cause of heterosis. Considerable research needs to be conducted with a variety of plant material and characters before the relative importance of these two phenomena in bringing about heterosis can be evaluated.

It will be remembered that Jones (*23*), in his hypothesis explaining why the corn breeders had failed to obtain inbred lines as vigorous as the F_1 generations, assumed linkage between genes favorable to growth and those not so favorable. Anderson (*1*), working with a cross between *Nicotiana alata* and *N. langsdorffii*, has published data that have a bearing upon this problem. He discussed the hindrances to free recombination of characters under the headings gametic elimination, zygotic elimination, "spurious pleiotropy," and linkage. In his material the two primary causes of gametic elimination were nonviability of pollen and genes for self-sterility. Anderson's (*1, p. 682*) comments concerning zygotic elimination in species crosses may be summed up by his statement:

> The complexion of any particular F_2 will be determined by the particular environment in which it was grown as well as by the possibilities inherent in the germ cells which begot it. Even under the so-called standard conditions of a scientific experiment there are numerous uncontrolled variables to which any two species will react differentially These will include such factors as time of year, sunshine, humidity, temperature, crowding, fumigation, and others. For every differentially selective factor in the environment there will tend to be a selection of F_2 segregates. An ideal F is as impossible as an ideal environment.

"Spurious pleiotropy" may be of considerable importance in all breeding work involving hybridization. For example, it is rather evident that any characters entirely dependent upon the same physiological genetic processes cannot be recombined. In regard to linkage, Anderson concludes that if the number of factors affecting a character equals or exceeds one per cross-over segment, then all such characters will be tightly linked, without regard to the further restrictions imposed by frequencies. From theoretical considerations he makes the following statement (*1, p. 689*):

> If we suppose that the species differ on the average by only four genes per crossover segment (which seems a ridiculously low value) then the total hindrance is in the neighborhood of 1/2048. This means that even though we were to grow an F_2 so large that it occupied all the arable land on the earth, we would still be obtaining less than 1/2000 of the gene combinations possible with no linkage. In our actual F_2 therefore we have been obtaining only a fraction of this fraction,

and in addition there have been the further restrictions imposed by pleiotropy and gametic and zygotic elimination.

Anderson (*1*) points out that his data are in agreement with the theoretical consideration. These studies further emphasize the difficulties one may expect to encounter in attempting to obtain inbred lines as vigorous as those F_1 generations exhibiting considerable heterosis.

NATURE OF THE INTERACTIONS OF GENES AS DETERMINED BY END PRODUCTS

The literature on the nature of the interactions of the genes as determined by end products is rather extensive, as in the broadest sense it includes both the physiological genetic and morphogenetic studies as well as other studies on quantitative inheritance. Also, much information can be obtained from papers on the applied phase of genetics, namely, plant and animal breeding. No attempt will be made to review all the literature having a bearing on this subject. Only a brief review covering some of the more important principles will be given. For a more extensive survey the reader is referred to Goldschmidt (*16*).

The theory of gene action as developed by Goldschmidt (*16*) has a direct bearing upon an interpretation of the data concerning the nature of the interactions of the genes differentiating quantitative characters, these interactions being determined by the measurement of end products. Goldschmidt pictures the development of the individual as due to reaction velocities in tune and postulates further that the reactions involved are catalyzed by genes. That the genes control rates of reactions (*15*, *48*) is basic to this theory. Other biological conceptions basic to Goldschmidt's theory of gene action are thresholds, timing, interrelationships of the different reactions catalyzed by the genes, and the numerical systems involved. A discussion of each of these phenomena has been given in previous publications (*16*, *37*) and need not be repeated here. However, since the present study has to do with the numerical systems involved, it may be well to review briefly some of the investigations pertaining to this phase of the matter

At least two numerical systems are of interest. (1) There may be no interactions between the genes affecting the quantitative character. Then, the effects of those genes will be arithmetically cumulative. This is well illustrated by the work of Mangelsdorf and Fraps (*34*), who found that in corn the vitamin A units per gram increased approximately 2.25 for each additional *Y* gene.

(2) The nature of the interactions of the genes affecting the quantitative character may be such that the effects of the genes are geometrically cumulative. The methods of attacking this problem have been quite diverse. From statistical considerations, Galton (*14*) expressed the belief that the forces acting on vital phenomena tend to have constant rate effects (effects in geometric progression). East (*9*) and Groth (*17*), from genetical and statistical considerations, came to the conclusion that in certain cases the effects of genes differentiating quantitative characters are geometrically cumulative. Lindstrom (*31*), working with crosses between species of *Lycopersicon*, clearly realized that this may be true. Houghtaling (*20*), from morphogenetic studies on the size of the tomato fruit, reached the same conclusion. Sinnott (*46*), MacArthur and Butler (*33*), and Charles and Smith (*3*), from a study of the means and frequency

distributions of the parents, and of the F_1 and F_2 generations, interpreted the results from size-inheritance studies as showing that the effects of the genes differentiating the quantitative characters are geometrically cumulative. Powers (*36*, *37*), by the use of marker genes, found that the nature of the interactions between the genes carried in different regions of the chromosome and the nature of the interactions between the factors represented by generations and those carried in the different regions of the chromosome were such that the effects of these genes were geometrically cumulative. The investigations of Houghtaling (*20*) and of MacArthur and Butler (*33*) show that size of fruit in the tomato is dependent upon at least three stages of development: Locule pattern formation, increase in cell number, and cell expansion. Since these stages of development occur in the sequence given, one might expect the effects of the genes to be geometrically cumulative. Powers (*37*) found that such was the case. He termed this an interstagic type of interaction of the genes. His work showed also that the intrastagic type of interaction of the genes pertaining to number of locules was such that the effects of the genes were geometrically cumulative.

EXPERIMENTAL CONSIDERATIONS

The experimental considerations in designing an experiment are dependent to a great extent on the objectives of the investigations. The objective of the present study was to obtain more information concerning the genetic phenomena of heterosis and dominance and concerning the nature of the interactions of the genes differentiating the quantitative characters.

Yield being a very important economic consideration in tomato production, the following characters affecting yield were selected for study: Weight per fruit, number of locules per fruit, number of fruits per centimeter of branch, height of plant, and spread of plant. It is apparent that weight of fruit and number of locules have economic importance other than through the influence they have upon yield. In obtaining weight of fruit data, 5 fruits per plant were weighed and averaged in 1938 and 10 fruits per plant in 1939. The weights were expressed in grams. As for number of locules the counts were based on 2 fruits per plant in 1938 and on 5 fruits per plant in 1939. To determine number of fruits per centimeter of branch, one representative main branch of each plant was measured for length and the fruits on that branch were counted; then the number of fruits was divided by the length of the branch expressed in centimeters. In measuring height of plant and spread of plant, care was taken to obtain measurements representative of the plant and to avoid extremes. Height is the distance from the ground level at the base of the plant to the top, and spread of the plant is the diameter measured through the main stem as the center. Both of these measurements are expressed in centimeters.

From genetic considerations and since the objective of the investigations was to obtain more information concerning the phenomena of heterosis and dominance and the nature of the interactions of the genes differentiating these characters, it is apparent that the parents, the F_1 generation, the F_2 generation, and the backcrosses to the respective parents should be included in the tests. All of this material was grown in a randomized-block experiment, there being a total of 20 blocks in each of the years 1938 and 1939. In 1938, two plots per block of each segregating generation were grown to one plot per block of the parents and F_1 generation. In 1939 only one plot of each was grown per block. The parents and generations were randomized within blocks, the randomization being the same for greenhouse propagation and field planting within any specified year. All operations such as transplanting were performed on the basis of blocks. Hence, since randomization was within blocks, any differences due to operations would not be confounded with the differences, such as those between generations, between parents, and between parents and generations, which the experiment was designed to study.

The parental material comprised the varieties Bonny Best, Danmark, and Johannisfeuer of *Lycopersicon esculentum* Mill. and the Red Currant variety of *L. pimpinellifolium* (Jusl.) Mill. The crosses Johannisfeuer × Danmark and Danmark × Red Currant were grown in 1938, and the crosses Johannisfeuer × Red Currant, Johannisfeuer × Bonny Best, and Johannisfeuer × Danmark were grown in 1939, Johannisfeuer × Danmark being grown during both years of the study. The purpose of the experimental design was to place the greatest emphasis on variety of plant material without entirely neglecting environmental influences as represented by years. The immediately foregoing discussion may be summarized as follows: Data for 5 quantitative characters were taken from somewhat more than 12,000 plants. The 12,000 plants represented 4 different crosses and were grown in a randomized block experiment composed of 20 blocks.

The methods employed in reducing the data were essentially the same as those given by Powers (*36*, *37*) with the exception of some minor changes, desirable because of the nature of the experiment. Only these changes need be mentioned here. Since the interest in this study lies primarily in comparisons between averages of parents and generations, the plot instead of the plant was used as the unit for reducing the data. Then, for each parent and generation the standard errors were estimated for within crosses and for within generations and parents on the basis of the averages of plots, these averages being derived from the individual plant data for the respective plots. In all tables the standard errors of the means are given. Differences having P values less than 0.05 were considered as statistically significant. The symbol B_1, which appears both in the tables and in the text, signifies that the progeny indicated resulted from backcrossing the F_1 to the designated parent.

The details of the experimental design are given in another paper (*38*) dealing with stages of development and need not be repeated here.

HETEROSIS AND DOMINANCE

THEORETICAL CONSIDERATIONS

When interpreting and evaluating the data, it is of decided benefit to have in mind the theoretically expected behavior of the generation means. First, the F_1 mean will be compared with the parental means to obtain information as to whether heterosis, complete dominance, partial dominance, or no dominance exists. With this information available, it is possible to predict where the means of the backcrossed generations and the mean of the F_2 generation should fall in respect to the mean of the F_1 and the two parental means. If the F_1 generation shows heterosis, at least one of the backcross generations should show heterosis also. This should be the backcross to the parent more closely approaching the F_1. The other backcross may or may not show heterosis, depending upon the disparity between the means of the parents. The mean of the F_2 should fall between the means of the backcross generations and would not be a very close approximation of the mean of the F_1. On the other hand, if there were no heterosis but instead some type of dominance or no dominance, theoretically the following behavior would be expected: The mean of either backcross generation should fall between the mean of the F_1 and the mean of the parent to which the backcross was made; and the mean of the F_2 should fall between the mean of the backcross generations, and it should approach more closely the mean of the F_1 as the degree of dominance became less. Hence, with no dominance, the F_2 mean should be a very close approximation of the F_1 mean. With these theoretical predictions in mind we are in a better position to interpret and evaluate the data.

EXPERIMENTAL DATA

The experimental results will be discussed under five headings: (1) Weight of fruits, (2) number of locules per fruit, (3) number of fruits per centimeter of branch, (4) plant height, and (5) plant spread.

Weight of Fruits

The means for weights of the individual fruits expressed in grams are given in table 1. For the cross Danmark × Johannisfeuer, in both 1938 and 1939, the mean of the F_1 generation falls between the means of the two parents but is closer to the mean of the smaller fruited parent, Johannisfeuer. The difference between the two differences involved in this latter comparison is statistically significant for 1938 but is not statistically significant for 1939. However, since these differences for both years are in the same direction, one can safely conclude that small size of fruit is partially dominant. The remaining data for this cross for 1938, aside from the fact that the means of the backcross generations and the mean of the F_2 fall between the means of the two parents, are not sufficiently consistent to allow any conclusion to be drawn either for or against the supposition that small size of fruit is partially dominant. The data for the cross Danmark × Johannisfeuer collected in 1939 and the data for the other crosses may be discussed together. In every case the mean of the F_1 lies closer to the mean of the smaller fruited parent.

For the crosses Danmark × Red Currant and Johannisfeuer × Red Currant the differences involved in the comparisons are statistically significant, whereas the differences involved in the comparisons for the crosses Danmark × Johannisfeuer and Johannisfeuer × Bonny Best do not reach statistical significance. In these crosses small size of fruit seems to be partially dominant. If such is the case, the following should be true: The mean of the backcross to the larger fruited parent should fall between the mean of the F_1 and the mean of the larger fruited parent; the mean of the backcross to the smaller fruited parent should fall between the mean of the F_1 and the mean of the smaller fruited parent; and the mean of the F_2 should fall between the means of the two backcross generations. The experimental data for Danmark × Johannisfeuer in 1939 and for the other three crosses are in general agreement with the theoretical expectation. Hence, from these data it may be concluded that small size of fruit is partially dominant for the tomato crosses involved.

TABLE 1.—*Means for weight of individual fruits*

Hybrid, generation, or parent	Mean weight of fruits in 1938	Mean weight of fruits in 1939	Hybrid, generation, or parent	Mean weight of fruits in 1939
Danmark × Johannisfeuer:	*Grams*	*Grams*	Johannisfeuer × Bonny Best:	*Grams*
Johannisfeuer	40.140±2.773	55.004±2.330	Johannisfeuer	55.004±2.330
B_1 to Johannisfeuer	45.975±1.918	64.040±2.200	B_1 to Johannisfeuer	63.826±2.816
F_1 generation	41.325±1.996	70.043±3.035	F_1 generation	82.019±3.119
F_2 generation	49.548±1.908	69.329±1.934	F_2 generation	81.336±3.817
B_1 to Danmark	43.970±1.810	78.079±2.405	B_1 to Bonny Best	90.736±3.011
Danmark	51.015±2.675	87.934±2.017	Bonny Best	119.616±3.037
Danmark × Red Currant:			Johannisfeuer × Red Currant:	
Red Currant	.915±.032		Red Currant	.903±.029
B_1 to Red Currant	2.095±.068		B_1 to Red Currant	2.26 ±.086
F_1 generation	5.480±.318		F_1 generation	6.682±.134
F_2 generation	5.380±.196		F_2 generation	6.066±.318
B_1 to Danmark	16.105±.572		B_1 to Johannisfeuer	18.948±.90
Danmark	51.170±3.518		Johannisfeuer	55.004±2.330

NUMBER OF LOCULES PER FRUIT

The means for number of locules per fruit are listed in table 2. The data for the Danmark × Johannisfeuer and the Johannisfeuer × Bonny Best crosses are very similar and hence may be discussed together. In no case is the F_1 mean significantly different from the mean of the fewer loculed parent. It would seem that small number of locules is completely dominant. This conclusion is substantiated by the means of the backcross to the fewer loculed parent. In fact, for 1939 the mean of the backcross to the fewer loculed parent in both of these crosses is smaller than the mean of the fewer loculed parent. The means of the backcrosses to the many-loculed parent in all cases fall between the mean of the F_1 and the mean of the many-loculed parent. The means of the F_2 in all cases fall between the means of the backcrosses, and approximate but are somewhat greater than the means of the F_1. The consistency of the data both for years and for crosses leads the writer to put considerable confidence in the conclusion that small number of locules is completely dominant in the crosses Danmark × Johannisfeuer and Johannisfeuer × Bonny Best.

TABLE 2.—*Means for number of locules per fruit*

Hybrid, generation, or parent	Mean locules per fruit in 1938	Mean locules per fruit in 1939	Hybrid, generation, or parent	Mean locules per fruit in 1939
Danmark × Johannisfeuer:	*Number*	*Number*	Johannisfeuer × Bonny Best:	*Number*
Danmark	5.475±0.057	6.183±0.085	Bonny Best	6.318±0.069
B_1 to Danmark	5.575±.064	5.898±.097	B_1 to Bonny Best	5.452±.077
F_1 generation	5.500±.086	6.051±.098	F_1 generation	6.329±.111
F_2 generation	6.595±.118	6.826±.150	F_2 generation	6.781±.208
B_1 to Johannisfeuer	7.500±.100	7.344±.136	B_1 to Johannisfeuer	7.031±.102
Johannisfeuer	9.125±.091	9.028±.084	Johannisfeuer	9.028±.084
Danmark × Red Currant:			Johannisfeuer × Red Currant:	
Red Currant	2.050±.014		Red Currant	2.034±.004
B_1 to Red Currant	2.205±.015		B_1 to Red Currant	2.183±.016
F_1 generation	2.395±.018		F_1 generation	2.517±.029
F_2 generation	2.570±.022		F_2 generation	2.886±.078
B_1 to Danmark	3.473±.037		B_1 to Johannisfeuer	4.356±.140
Danmark	5.405±.008		Johannisfeuer	9.028±.084

Likewise, the data for the crosses Danmark × Red Currant and Johannisfeuer × Red Currant are very similar and therefore may be discussed together. The F_1 means lie between the means of the respective parents, but in both cases materially closer to the mean of the fewer loculed parent. It would appear that small number of locules is partially dominant. The means of the backcrosses to the parents having smaller number of locules lie between the F_1 means and the means of the parents having smaller number of locules. The means of the other backcrosses lie between the F_1 means and the means of the parents having a greater number of locules. The means of the F_2 fall between the means of the backcross generations and approach the F_1 means rather closely but are somewhat larger. The data for Danmark × Red Currant and Johannisfeuer × Red Currant are in agreement with the conclusion that small number of locules is partially dominant. Again the consistency of the data allows one to have considerable confidence in the validity of this deduction.

NUMBER OF FRUITS PER CENTIMETER OF BRANCH

The means for number of fruits per centimeter of branch are given in table 3. First, consider the cross Danmark × Johannisfeuer. The mean of the F_1 for 1938 falls between the means of the parents, indicating partial dominance of fewer fruits per centimeter of branch. The means of the backcrosses and the mean of the F_2 support this conclusion. For this same cross in 1939 the mean of the F_1 and the mean of the backcross to Johannisfeuer are not significantly different from the mean of Johannisfeuer, the parent having smaller number of fruits. This would indicate complete dominance of small number of fruits per centimeter of branch. The mean of the F_2 and the mean of the backcross to the parent having a greater number of fruits per centimeter of branch are in agreement with this conclusion.

For the cross Johannisfeuer × Bonny Best the difference between the means of the parents is not very great. The fact that the means of all the hybrid generations, including the F_1, fall between the means of the two parents is rather conclusive evidence that dominance is partial.

TABLE 3.—*Means for number of fruits per centimeter of branch*

Hybrid generation or parent	Mean fruit per cm branch in 1938	Mean fruit per cm branch in 1939
Danmark × Johannisfeuer	*Number*	*Number*
Johannisfeuer	0.216±0.008	0.220±0.009
B_1 to Johannisfeuer	.22 ±.004	.201±.008
F_1 generation	.243±.007	.211±.007
F_2 generation	.261±.008	.239±.010
B_1 to Danmark	.320±.00[illegible]	.2[illegible]4±.012
Danmark	.410±.012	.337±.014
Danmark × Red Currant		
Danmark	.417±.012	
B_1 to Danmark	.404±.010	
F_1 generation	.359±.009	
F_2 generation	.428±.011	
B_1 to Red Currant	.423±.011	
Red Currant	.441±.017	

Hybrid generation or parent	Mean fruits per cm branch in 1939
Johannisfeuer × Bonny Best	*Number*
Bonny Best	0.187±0.008
B_1 to Bonny Best	.197±.010
F_1 generation	.192±.007
F_2 generation	.191±.008
B_1 to Johannisfeuer	.197±.007
Johannisfeuer	.2[illegible]0±.009
Johannisfeuer × Red Currant	
Johannisfeuer	.220±.009
B_1 to Johannisfeuer	.211±.009
F_1 generation	.302±.015
F_2 generation	.30[illegible]±.01[illegible]
B_1 to Red Currant	.393±.024
Red Currant	.[illegible]48±.030

Considering the F_1 for the cross Danmark × Red Currant, the following facts regarding number of fruits per centimeter of branch become apparent. The mean of the F_1 is smaller than the mean of either parent; the mean of the backcross to Danmark falls between the mean of the F_1 and the mean of Danmark; and the mean of the F_2 and the mean of the backcross to Red Currant fall between the mean of the F_1 and the mean of the Red Currant parent. These facts are typical of heterosis. That this is an expression of heterosis is apparent from a consideration of the length of internode as determined by the distance between leaf axils in the F_1 as compared with the same character in the parents. The distance between leaf axils was considerably greater in the F_1 than was the distance between leaf axils in either parent. This fact is reflected in the spread and height data, which will be given later. The greater length of the internodes of the F_1 would result in a greater number of centimeters per cluster and would be expected to result in fewer fruits per centimeter of branch length. Accordingly, the heterosis noted is probably due to the vegetative vigor of the plants.

For the cross Johannisfeuer × Red Currant, the F_1 mean falls between the means of the two parents; the mean of the backcross to the Johannisfeuer parent falls between the means of the F_1 and of the Johannisfeuer parent; and the mean of the backcross to the Red Currant parent and the F_2 mean fall between the means of the F_1 and of the Red Currant parent. Hence, fewer fruits per centimeter of branch length is partially dominant.

These data clearly demonstrate that heterosis and dominance, insofar as inheritance of the character number of fruits per centimeter of branch is concerned, are dependent upon both the cross and the environmental conditions as represented by years.

PLANT HEIGHT

The means for plant height, expressed in centimeters, are listed in table 4. The crosses Danmark × Johannisfeuer, Danmark × Red Currant, and Johannisfeuer × Red Currant may be discussed together. In these cases the means of the F_1 exceed the means of all other hybrid generations and the means of the parents. Moreover, in all of these crosses the means of the backcross generations fall between their respective F_1 means and those of the parents to which they were backcrossed. In all these crosses the means of the F_2 generations fall between the means of their respective backcross generations. One other fact concerning the data should be noted. With the exception of the B_1 to Danmark mean in 1939 (for the cross Danmark × Johannisfeuer) all of the hybrid generation means exceed the means of their respective parents. From these data it may be concluded that all of these crosses show heterosis for plant height.

For the cross Johannisfeuer × Bonny Best, the F_1 mean falls between the means of the two parents, but slightly closer to the mean of the taller parent. The difference involving two differences (F_1—Johannisfeuer)—(Bonny Best—F_1), in this comparison is not statistically significant. For the cross Johannisfeuer × Bonny Best, plant height approaches intermediacy or no dominance. The magnitudes of the means for the other hybrid generations are in accord with these conclusions.

TABLE 4.—*Means for plant height*

Hybrid, generation, or parent	Mean plant height in 1938	Mean plant height in 1939	Hybrid, generation, or parent	Mean plant height in 1939
Danmark × Johannisfeuer	*Centimeters*	*Centimeters*	Johannisfeuer × Bonny Best	*Centimeters*
Danmark	35.40±0.617	35.57±0.127	Johannisfeuer	39.43±0.495
B_1 to Danmark	39.38±.687	38.45±.480	B_1 to Johannisfeuer	41.66±.675
F_1 generation	43.80±.912	41.21±.677	F_1 generation	43.67±.606
F_2 generation	41.48±.622	39.56±.740	F_2 generation	43.61±.868
B_1 to Johannisfeuer	41.90±.683	41.07±.516	B_1 to Bonny Best	45.28±.566
Johannisfeuer	38.95±.851	39.43±.495	Bonny Best	46.87±.556
Danmark × Red Currant			Johannisfeuer × Red Currant	
Red Currant	27.90±.676		Red Currant	30.79±.665
B_1 to Red Currant	37.73±.666		B_1 to Red Currant	39.77±.762
F_1 generation	44.45±1.202		F_1 generation	43.19±.832
F_2 generation	35.93±.616		F_2 generation	39.93±.855
B_1 to Danmark	38.88±.600		B_1 to Johannisfeuer	40.58±.524
Danmark	34.87±.701		Johannisfeuer	39.43±.495

PLANT SPREAD

The means for plant spread, expressed in centimeters, are given in table 5. The data for all of the crosses may be considered together. Without exception the means of the F_1 generations exceeded the means of all other hybrid generations and the means of the parents in their respective crosses. Also, in every cross the mean of the backcross to the parent with the greatest spread exceeded the mean of the parent with the greatest spread. The means of the backcrosses to the parents with the smaller spread, in all cases, fell between the means of the respective F_1 generations and those of the parents with the smaller spread. Therefore, we may conclude that all crosses show heterosis for plant spread, heterosis being particularly pronounced in crosses including Red Currant.

TABLE 5.—*Means for plant spread*

Hybrid, generation, or parent	Mean plant spread in 1938	Mean plant spread in 1939	Hybrid, generation, or parent	Mean plant spread in 1939
Danmark × Johannisfeuer	*Centimeters*	*Centimeters*	Johannisfeuer × Bonny Best	*Centimeters*
Danmark	66.65±1.916	107.15±2.651	Bonny Best	128.05±1.605
B_1 to Danmark	77.05±2.233	130.46±1.938	B_1 to Bonny Best	139.52±2.151
F_1 generation	94.70±4.479	155.32±1.625	F_1 generation	151.06±1.933
F_2 generation	84.25±2.183	139.65±2.620	F_2 generation	146.47±1.970
B_1 to Johannisfeuer	88.15±2.661	153.49±1.990	B_1 to Johannisfeuer	147.73±2.056
Johannisfeuer	79.20±3.509	144.59±2.103	Johannisfeuer	144.59±2.103
Danmark × Red Currant			Johannisfeuer × Red Currant	
Danmark	66.85±2.263		Johannisfeuer	144.59±2.103
B_1 to Danmark	93.68±2.294		B_1 to Johannisfeuer	173.13±2.597
F_1 generation	137.57±4.913		F_1 generation	198.38±2.202
F_2 generation	105.98±2.720		F_2 generation	173.07±2.113
B_1 to Red Currant	120.68±3.531		B_1 to Red Currant	179.46±1.980
Red Currant	106.65±1.475		Red Currant	159.98±1.302

NATURE OF INTERACTIONS OF GENES

THEORETICAL CONSIDERATIONS

The data concerning the nature of the interactions of the genes have a bearing upon two of the possible numerical systems involved. It is important from the standpoint of both genetics and plant breeding to know whether the effects of the genes are arithmetically or geometrically cumulative. The formulas used in obtaining the theoretical means are the same as those described by Powers and Lyon (*38*) and need not be repeated here. As previously pointed out (*38*), the method employing these formulas is not always discriminative. This was found to be true in the case of many of the data collected in this study. In fact, the method was found to be discriminative for only part of the data on weight of individual fruits and for only part of those on number of locules.

EXPERIMENTAL DATA

WEIGHT OF FRUITS

The obtained and theoretical means for weight of individual fruits are given in table 6. Very little if any information can be obtained concerning the nature of the interactions of the genes for the crosses Danmark × Johannisfeuer and Johannisfeuer × Bonny Best, because the two sets of theoretical means are very nearly equal. However, such is not the case with the crosses Danmark × Red Currant and Johannisfeuer × Red Currant. There are only very small differences between the obtained means and the theoretical means calculated on the assumption that the effects of the genes differentiating weight of fruit are geometrically cumulative. It is rather apparent that the fit is very poor between the obtained means and those calculated on the assumption that the effects of the genes differentiating weight of fruit are arithmetically cumulative. Even though the fit is fairly good between the obtained means and the theoretical means based on

TABLE 6.—*Obtained and theoretical means for weight of individual fruits*

Hybrid, year, and generation	Mean weight of fruits		
	Obtained	Theoretical	
		Geometric	Arithmetic
Danmark × Johannisfeuer			
1938	*Grams*	*Grams*	*Grams*
B_1 to Johannisfeuer	45.975±1.918	40.723	40.728±1.717
F_2 generation	49.548±1.908	43.863	44.199±1.411
B_1 to Danmark	43.970±1.810	47.216	47.670±1.631
1939			
B_1 to Johannisfeuer	64.010±2.260	62.070	62.524±1.881
F_2 generation	69.329±1.934	69.794	70.756±1.730
B_1 Danmark	78.079±2.405	78.480	78.989±1.924
Johannisfeuer × Bonny Best			
1939			
B_1 to Johannisfeuer	63.826±2.816	67.167	68.512±1.912
F_2 generation	81.336±3.847	81.565	84.665±1.849
B_1 to Bonny Best	90.736±3.011	99.049	100.818±2.264
Danmark × Red Currant			
1938			
B_1 to Red Currant	2.095±.068	2.239	3.198±.076
F_2 generation	5.380±.106	6.121	15.761±.211
B_1 to Danmark	16.105±.552	16.745	28.325±.757
Johannisfeuer × Red Currant			
1939			
B_1 to Red Currant	2.265±.086	2.456	3.793±.046
F_2 generation	6.066±.318	6.862	17.318±.114
B_1 to Johannisfeuer	18.948±.805	19.171	30.618±.419

the geometrically cumulative hypothesis, it should be noted that in both crosses these theoretical means are consistently larger than the obtained means. It is quite evident that these differences cannot be attributed to the errors of random sampling. One cannot draw the conclusion from these data that the effects of the genes differentiating weight of fruit are strictly geometrically cumulative. Some unknown quantity is exerting an influence.

NUMBER OF LOCULES PER FRUIT

The obtained and theoretical means for number of locules are given in table 7. Again for the crosses Danmark × Johannisfeuer and Johannisfeuer × Bonny Best, a comparison between obtained and theoretical means is of little value in determining the nature of the interactions of the genes as determined by measurements of end products. Again the difficulty lies in the nondiscriminative nature of the method employed. However, such is not the case with the crosses Danmark × Red Currant and Johannisfeuer × Red Currant. The fit between the obtained means and those calculated on the assumption that the effects of the genes are arithmetically cumulative is not good. The fit between the obtained means and the means calculated on the assumption that the effects of the genes are geometrically cumulative is considerably closer. But again the calculated means for both crosses are considerably larger than the obtained means; and again it is apparent that some unknown quantity or quantities are influencing

TABLE 7.—*Obtained and theoretical means for number of locules*

Hybrid, year and generation	Mean locules per fruit		
	Obtained	Theoretical	
		Geometric	Arithmetic
Danmark × Johannisfeuer			
1938	*Number*	*Number*	*Number*
B₁ to Danmark	5.575±0.064	5.487	5.488±0.052
F₁ generation	6.595± .118	6.235	6.400± .051
B₁ to Johannisfeuer	7.500± .100	7.081	7.313± .066
1939			
B₁ to Danmark	5.898± .097	6.117	6.117± .058
F₁ generation	6.826± .150	6.724	6.828± .058
B₁ to Johannisfeuer	7.344± .136	7.391	7.510± .068
Johannisfeuer × Bonny Best			
1939			
B₁ to Bonny Best	5.452± .077	6.323	6.324± .065
F₁ generation	6.781± .208	6.914	7.001± .066
B₁ to Johannisfeuer	7.034± .162	7.559	7.679± .075
Danmark × Red Currant			
1938			
B₁ to Red Currant	2.205± .015	2.216	2.223± .011
F₁ generation	2.570± .022	2.824	3.011± .015
B₁ to Danmark	3.473± .037	3.598	3.900± .026
Johannisfeuer × Red Currant			
1939			
B₁ to Red Currant	2.183± .016	2.263	2.276± .013
F₁ generation	2.886± .078	3.284	4.021± .020
B₁ to Johannisfeuer	4.356± .140	4.767	5.773± .035

the results. From these data we cannot safely conclude that the effects of the genes are strictly geometrically cumulative. By the use of marker genes Powers (*36*, *37*) has shown that in the *Lycopersicon* material studied the results were those expected on the theory that the effects of the interactions of the genes influencing both weight of fruit and number of locules are geometrically cumulative.

DISCUSSION AND CONCLUSIONS

HETEROSIS AND DOMINANCE

Plant-Breeding Methods Utilizing Heterosis and Dominance

Before taking up the relation that the foregoing data have to a tomato-breeding program, it may be well to consider some of the plant-breeding methods that have been developed to utilize heterosis and dominance. These methods include the following: (1) Inbreeding, followed by hybridization; (2) production of F_1 hybrids, three-way hybrids, double hybrids, advanced-generation hybrids, and inbred line × variety hybrids; (3) production of synthetic varieties; (4) predicting yields of hybrids from inbred lines; (5) improvement of inbred lines.

Shull (*42*, *43*, *44*, *45*), East (*6*, *7*, *8*), Jones (*23*, *24*), and East and Hayes (*11*) did the early work leading to the modern methods of breeding crops in which the utilization of heterosis is a primary consideration. They demonstrated the advantages of inbreeding followed by hybridization. Hence, the production of inbred lines and the determination of the combining ability (ability to produce high-yielding hybrids) of these inbred lines became a standard procedure for corn breeders.

Problems concerning the most efficient means of utilizing these inbred lines soon arose. Shull (*43*) was the first to suggest growing the F_1 generation between two inbred lines of corn to produce the commercial crop. However, the poor yield and quality of seed produced by inbred lines of corn are obstacles to the use of F_1 hybrids. To overcome these difficulties Jones (*24*) proposed the use of double crosses. Soon after this, three-way crosses came into use. Jones' idea was to cross two F_1 generations of different inbred parentage and to use seed thus produced for planting the commercial crop. This method has proved very practical and is now in general use throughout the United States. In 1930 Kiesselbach (*26*) published results showing that yields from advanced-generation hybrids were equal to those from the original double cross. As the name implies, this method involves crosses between generations subsequent to the F_1. For example, the two F_2 generations obtained from the F_1 generations making up the original double cross would be hybridized to produce an advanced-generation double cross. The use of inbred line × variety hybrids for the production of the commercial crop was first suggested by Lindstrom (*30*). This method involves crossing an inbred line and a variety, and in some cases satisfactory yields have been obtained.

All of the foregoing methods developed to utilize heterosis in corn production have a common disadvantage, namely, that the farmer cannot save seed for the production of next year's crop from the current crop. To overcome this difficulty Hayes and Garber (*19*) suggested using inbred lines to produce synthetic varieties. So far no outstanding synthetic varieties have been produced. It seems that synthetic varieties offer promise, and hence the production of such varieties merits more consideration than it has received in the past. This fact is emphasized by the findings of Wright (*49*), Neal (*35*), and Kiesselbach (*26*). Wright (*49*) has shown that "a random-bred stock derived from *n* inbred families will have 1/*n*th less superiority over its inbred ancestry than the first cross or a random-bred stock from which the inbred families might have been derived without selection." Neal (*35*) found that for 10 single hybrids, 4 three-way hybrids, and 10 double hybrids the agreement was very good between the obtained and theoretical means (calculated by the use of Wright's formula). Kiesselbach (*27*), in 1933, reported the results of tests made over a period of years to determine the comparative amounts of yield reduction in the second generation of various kinds of crosses, including single, double, 8-line, and 16-line hybrids. He reached the following conclusions:

> The decrease in yield of any hybrid in the F_2 generation is equal to half the difference in yield between its F_1 and the mean yield of the open pollinated parents. This will also apply to any advanced generation in the absence of selection. This reduced yield is due to a reduction in the number of favorable growth factors as a consequence of close breeding. In any orthodox hybrid in which the two parents are regularly built up from equal numbers of lines this reduction tends to be inversely proportional to the number of lines involved.

Thus Wright (*49*), working with animals, and Kiesselbach (*27*), working with plants, drew similar conclusions. From these investigations it seems rather safe to conclude that the amount of decrease in yield of grain in *Zea mays* accompanying inbreeding may be predicted by Wright's (*49*) formula, and therefore it should be possible to produce a high-yielding synthetic variety, provided a sufficient number of inbred lines having high combining ability are available.

One of the biggest problems of the corn breeder is ascertaining the combining ability of inbred lines when used in single, three-way, and double crosses. The obvious method is to make the possible crosses between the inbred lines and then test these crosses for yielding ability. Where large numbers of inbred lines are available, this method is almost prohibitive owing to the large amount of labor, time, and land required for testing. Therefore, methods materially reducing these requirements are needed. Jenkins and Brunson (*22*) pointed out that, for producing double crosses, multiple crosses, or synthetic varieties, the most valuable lines are those that cross well with a large number of inbred lines, i. e., those that produce, on the average, the best hybrids when tested with a rather wide range of germ plasm. Jenkins and Brunson (*22*) also reported the results of studies to determine whether this wide range of germ plasm might not be supplied by crossing with a mixed collection of varieties, or possibly by crossing with an individual variety. From these studies they concluded that crosses with open-pollinated varieties may be used efficiently in the preliminary testing of new lines. Following up this work, Jenkins (*21*) found that the information obtained from comparisons of inbred-line variety crosses may be utilized to advantage in estimating the performance of double crosses among these lines. Jenkins (*21*) also gave a method of estimating the yields of double crosses, based on genetic considerations. He pointed out that

> In any double cross the genes of each of the four parental lines are united only with allelomorphs of the two lines which entered the double cross from the opposite

parent. In the double cross $WX \times YZ$, therefore, it would seem that the crosses WY, WZ, XY, and XZ better represent the hybrid combinations actually occurring than do all six of the possible combinations among these lines.

Following this suggestion, Doxtator and Johnson (*5*) demonstrated that highly significant differences in yielding ability can be found in double crosses resulting from the use of single-cross parents produced from four inbred lines, and that by the appropriate use of single-cross data the highest yielding double-cross combination may be predicted. Hence, it would seem that the methods developed by Jenkins (*21*) would greatly expedite testing the combining ability of inbred lines.

From the foregoing discussions, it is apparent that the improvement of inbred lines, particularly as to yielding ability and quality of seed, would aid materially in solving many of the corn-breeding problems. Convergent improvement was developed by Richey (*39*) as a method of increasing the yielding ability of inbred lines and at the same time maintaining their combining ability with certain other inbred lines. The procedure for this method was as follows: Two inbred lines that combined exceptionally well were crossed to produce an F_1 generation; then the F_1 generation was backcrossed to each inbred line; selection for vigor and characters of the nonrecurring parent was practiced in these two series of backcrosses; these selections of any one series were again backcrossed to the inbred line occurring in that series, and again, from the progeny, individuals possessing vigor and other desirable characters of the nonrecurring parent were selected. This procedure with the two series of backcrosses was continued until the yields of the crosses between the selected lines and the nonrecurring parent equaled or exceeded the yields of the F_1 generation obtained from crossing the original inbred lines. Richey and Sprague (*40*) found that three or four backcrossings were sufficient to produce hybrids whose yields were approximately equal to the yields of the F_1 crosses between the foundation inbred lines. Moreover, in these backcrossed generations there was indicated a permanent improvement of 13 to 15 percent over the recurring parent after allowing for differences in the degree of inbreeding. Then, as Richey and Sprague (*40*) pointed out, inasmuch as this excess presumably is due to the retention of additional dominant genes from the nonrecurring parent, as a result of selection among the progeny resulting from back-pollinating, the recovered lines are heterozygous for these genes and hence must be inbred to put at least some of these genes in the homozygous condition. After this has been accomplished the breeder is in a position to start a second cycle of convergent improvement. In summarizing the results from their tests with corn, Richey and Sprague (*40*) drew the following conclusions: In addition to the larger yields of the recovered lines, improvement has been achieved in ability to resist lodging and in the amount of pollen shed; yellow endosperm has been substituted for white and clear pericarp for red, all without changing significantly the behavior of the lines in crosses; convergent improvement, suggested originally from theoretical considerations as a means of improving selfed lines of corn without interfering with their behavior in hybrid combination, so far has been found successful; the results suggest that this method also may provide a means by which the yields of F_1 crosses between selfed lines can be raised to an even higher level. From the results obtained by Richey and Sprague, it seems safe to conclude that convergent improvement is a practical method of improving yielding ability and other desirable characters of inbred lines.

APPLICATION OF HETEROSIS AND DOMINANCE TO TOMATO BREEDING

In a tomato-breeding program it is apparent that the standard methods of breeding self-fertilized crops can and should be used. However, it is not so apparent that the methods developed for the improvement of cross-fertilized crops can be used advantageously in a tomato-breeding program. Information concerning heterosis and dominance of the more important economic characters of tomatoes is essential to the solution of this problem. The data reported in this paper give some of this information.

Small size of fruit, as determined by weight, was found to be partially dominant. Nearly the same situation exists in regard to number of locules, small number of locules being either completely or partially dominant. Partial dominance or heterosis was exhibited for smaller number of fruits per centimeter of branch. From these results it would seem that the tomato breeder cannot utilize heterosis to increase size of fruit, number of locules, or number of fruits per centimeter of branch. However, if smaller fruits or fruits with a smaller number of locules are desired, these can be obtained in hybrids by crossing with varieties or strains possessing these characters. Heterosis for increased height and spread of plant was obtained. Also, it will be remembered (*38*) that some of the crosses discussed in this paper exhibited decided heterosis for earliness of maturity. This was particularly true for those crosses having Red Currant as one of the parents. In regard to the characters studied, it may be concluded that the greatest benefits due to heterosis will come from increased plant size and increased earliness. Some of the increase in plant size should result in increased plant yields. It seems that increases in plant size and earliness are sufficient to warrant an intensive study of the possibility of utilizing heterosis in the commercial production of tomatoes.

These considerations indicate that the tomato breeder should employ those fundamental principles established by geneticists and breeders who have already attempted to take advantage of heterosis. For example, in building up inbred lines and varieties to be used in crosses, foundation stock as genetically diverse as possible should be used. The decided heterosis for increased plant size and for increased earliness exhibited by the crosses between *Lycopersicon esculentum* and *L. pimpinellifolium* amply demonstrates the advantages of such a method. During the development of these inbred lines and varieties the breeder and geneticist should not forget the necessity of obtaining information concerning the inheritance of the economically important characters. The breeding program can be pursued with much less expense and with much more certainty of success if information concerning the inheritance of these characters is available. The truth of this assertion is verified by the fact that all of the important advances in methods of corn breeding have been based on fundamental genetic principles. Also, the value of genetic information is well illustrated by the studies concerning weight of fruit, number of locules per fruit, and number of fruits per centimeter of branch length reported in this article. Since small weight of fruit, small number of locules, and small number of fruits per centimeter of branch are at least partially dominant, it is evident that the requirements desired in the commercial product should be striven for in the inbred lines, especially if *L. pimpinellifolium* has been used as foundation stock. On the other hand, such characters as earliness and size of plant can be obtained by taking advantage of heterosis, provided the inbred lines or varieties used in the crosses possess good combining ability. It is very apparent that further advances in methods of breeding and the utilization of the methods we now have will be dependent upon genetic information concerning the crop and knowledge of fundamental genetic principles.

In building up strains of tomatoes to be used in crosses, the backcross method and Richey's (*39*) convergent-improvement method will undoubtedly be of value. In using these methods caution should be exercised in order that material too similar genetically does not result. In certain material, to avoid this danger inherent in the backcross method, selection within F_2 and later generations could be practiced to advantage.

RELATION OF HETEROSIS AND DOMINANCE

In this paper it has been assumed that from the standpoint of physiological genetics there is no fundamental difference between the phenomena of heterosis and dominance. Some of the data reported in a previous article (*38*) pertain to this assumption. These data are presented in tables 8 and 9.

TABLE 8. *Means of number of days from seeding to first complete change of color of any fruit for the cross Danmark × Johannisfeuer*

neration or parent	Mean period from first complete ch	
	Days	*Days*
Johannisfeuer	161.9±0.900	135.1±0.838
B_1 to Johannisfeuer	165.0±0.463	133.3±0.787
F_1 generation	165.6±0.617	132.4±0.440
F_2 generation	166.4±0.137	134.9±0.727
B_1 to Danmark	167.6±0.382	134.8±0.983
Danmark	170.0±0.489	137.7±0.632

TABLE 9. *Means of number of days from first fruit set to first complete change of color of any fruit for 1939*

Hybrid, generation, or parent	Mean period from first fruit set to first complete change of color	Hybrid, generation, or parent	Mean period from first fruit set to first complete change of color
Johannisfeuer × Red Currant	*Days*	Danmark × Johannisfeuer	*Days*
Johannisfeuer	45.2±0.483	Johannisfeuer	45.2±0.483
B_1 to Johannisfeuer	44.0±0.498	B_1 to Johannisfeuer	47.6±0.564
F_1 generation	43.5±0.472	F_1 generation	48.0±0.516
F_2 generation	44.5±0.608	F_2 generation	49.9±0.412
B_1 to Red Currant	47.1±0.585	B_1 to Danmark	50.3±0.621
Red Currant	49.3±0.501	Danmark	54.6±0.563

From table 8 it can be seen that there are no significant differences between the 1938 means of number of days from seeding to first complete change of color in the case of Johannisfeuer, the B_1 to Johannisfeuer, and the F_1 generation. This shows that for 1938 smaller number of days from seeding to first complete change of color of any fruit is completely dominant. In 1939 the means of the F_1 generation and of the B_1 to Johannisfeuer were significantly smaller than the means of Johannisfeuer and of Danmark. Hence, smaller number of days from seeding to first complete change of color showed heterosis in 1939. Since the genotypes were constant for the two years, it is evident that the environment as represented by years determined whether the character should exhibit heterosis or dominance.

The means of number of days from first fruit set to first complete change of color of any fruit are listed in table 9. From this table it can be seen that for Johannisfeuer × Red Currant the means of the F_1, the B_1 to Johannisfeuer, and the F_2 generations are smaller than the mean of either parent. In these comparisons, the differences involving the mean of the F_1 are statistically significant. From these facts it may be concluded that the character smaller number of days from first fruit set to first complete change of color of any fruit exhibits heterosis. For Danmark × Johannisfeuer the means of all the generations are between the means of the two parents. Clearly smaller number of days from first fruit set to first complete change of color of any fruit is partially dominant. Since in this case the environmental influence has been controlled (randomized-block experiment with 20 replications) we may conclude that whether the character small number of days from first fruit set to first complete change of color of any fruit exhibits heterosis or dominance is determined by the genotype.

Hence, as the foregoing data indicate, in one case the environment and in another case the genotype determined whether a character should exhibit heterosis or partial dominance. This evidence is rather conclusive in support of the supposition that heterosis and dominance represent degrees of identical phenomena and are dependent upon the same physiological genetic processes.

NATURE OF INTERACTIONS OF GENES

Although genetics has contributed much to the advancement of plant breeding, there is need for considerably more work on the inheritance of the quantitative characters. This is particularly true for studies dealing with the nature of the interaction of the genes affecting the quantitative characters.

The corn breeders have accumulated considerable data having a bearing upon the nature of the interaction of the genes as determined by the measurement of an end product, namely, yield. Their data indicate that the effects of the genes differentiating yield in corn are arithmetically cumulative. In fact those formulas used in predicting theoretical decreases in yield accompanying inbreeding (*27, 35, 40, 49*) are based on the assumption that the effects of the genes differentiating yield are arithmetically cumulative. The fact that such good fits between the obtained and theoretical means were secured by these formulas would indicate that this assumption is correct. However, before such a conclusion is drawn tests should be made to determine whether the formulas are discriminative. In other words, would the obtained and theoretical data fit just as well if the theoretical calculations had been made on the assumption that the effects of the genes differentiating the quantitative characters are geometrically cumulative? To answer this question the theoretical F_2 means were calculated from Neal's (*35*) data for corn by using the formulas given in table 10. These theoretical means, together with the obtained means, are listed in table 11.

TABLE 10.—*Formulas for calculating theoretical F_2 mean yields of Zea mays from Neal's (35) data*

Hybrids	Arithmetic mean	Geometric mean
Single	$\frac{2\overline{F}_1+\overline{P}_1+\overline{P}}{4}$	Antilogarithm of $\left(\frac{2\log\overline{F}_1+\log\overline{P}_1+\log\overline{P}}{4}\right)$
3 way	$\frac{6\overline{F}_1+\overline{P}_1+\overline{P}_2+\overline{P}_3}{9}$	Antilogarithm of $\left(\frac{6\log\overline{F}_1+\log\overline{P}_1+\log\overline{P}_2+\log\overline{P}_3}{9}\right)$
Double	$\frac{12\overline{F}_1+\overline{P}_1+\overline{P}_2+\overline{P}_3+\overline{P}_4}{16}$	Antilogarithm of $\left(\frac{12\log\overline{F}_1+\log\overline{P}_1+\log\overline{P}_2+\log\overline{P}_3+\log\overline{P}_4}{16}\right)$

TABLE 11.—*Obtained and theoretical F_2 mean yields of Zea mays, the calculations being based on Neal's (35) data*

F₂ MEAN YIELDS OF ZEA MAYS

Cross	F_2 mean yield			Cross	F_2 mean yield			Cross	F_2 mean yield		
	Obtained	Arithmetic	Geometric		Obtained	Arithmetic	Geometric		Obtained	Arithmetic	Geometric
Single hybrid	*Bu*	*Bu*	*Bu*	3 way hybrid	*Bu*	*Bu*	*Bu*	Double hybrid (con.)	*Bu*	*Bu*	*Bu*
$3\times R_3$	45.7	40.4	35.6	$(R_3\times 3)\times 25$	49.0	51.7	46.2	$(R_3\times 6)\times(3\times 25)$	55.1	[illegible]	[illegible]
$R_3\times 6$	47.0	43.0	38.5	$(3\times 25)\times R_3$	44.7	48.1	43.7	$(23\times R_3)\times(M_{13}\times 6)$	52.6	54.4	50.5
$R_3\times 25$	43.7	41.5	36.3	$(R_3\times 25)\times 6$	56.9	54.4	48.9	$(3\times 6)\times(23\times 25)$	57.4	55.2	51.9
$R_3\times 26$	34.8	38.5	34.4	$(6\times 25)\times R_3$	46.5	48.8	44.9	$(R_3\times 25)\times(M_{13}\times 23)$	45.2	52.9	49.0
3×25	41.7	47.7	41.7	Double hybrid				$(3\times R_3)\times(6\times 23)$	56.9	55.6	51.6
3×26	44.7	46.3	40.8	$(R_3\times 6)\times(23\times 25)$	52.0	54.7	50.9				
6×26	52.6	47.9	43.4	$(M_{13}\times R_3)\times(6\times 25)$	50.0	49.6	46.5				
$M_{13}\times R_3$	40.9	39.7	34.9	$(3\times R_3)\times(23\times 25)$	58.[illegible]	57.1	52.3				
$R_3\times 23$	41.5	41.6	37.0	$(6\times 25)\times(3\times R_3)$	58.2	56.9	52.4				
23×26	46.5	46.5	41.7	$(23\times R_3)\times(3\times 25)$	53.6	51.4	47.9				

ANALYSIS SUMMARY OF SINGLE HYBRIDS FOR OBTAINED AND ARITHMETIC MEANS

Source of variation	Degrees of freedom	Sum of squares	Variance	F value	P value[1]
Crosses	9	239.908000	26.656444	3.837914	0.05
Means	1	4.050000	4.050000	.583107	.05
Crosses × means	9	62.510000	6.945556		
Total	19	306.468000			

[1] The P values for determining the fit between the obtained and arithmetic means were greater than 0.05. The P values for determining the fit between the obtained and geometric means were less than 0.01.

Some explanation concerning the method of treatment of these data should be given. To determine whether the obtained means were in agreement with either group of the theoretical means, the analysis of variance was applied to each group of hybrids. The method used is shown at the bottom of table 11. The data included in this illustration are the means obtained for the single hybrids and the means calculated on the assumption that the effects of the genes differentiating yields are arithmetically cumulative. In using this method one should examine the detailed data to ascertain whether a few extreme deviates occur. If this is done there should be no misinterpretation of the data.

From table 11 it can be seen that the agreement is generally good between the obtained data and the theoretical data calculated on the assumption that the effects of the genes differentiating yield in these inbred lines of corn are arithmetically cumulative. On the other hand, the disparity between the obtained data and those calculated on the assumption that the effects of the genes are geometrically cumulative is in general too great to be accounted for by the probable errors of random sampling. These findings are supported by the data of Richey and Sprague (*40*) and by those of Kiesselbach (*27*). It should be pointed out that in applying the above formulas to data for three-way and double hybrids, if the possible F_1 hybrids differ materially in yield the data from the highest yielding combination (*5, 21*) should be used; otherwise erroneous conclusions may be drawn. Where the possible F_1 hybrids do not differ greatly in yield this consideration seems to be merely academic.

It will be remembered that in the present study with tomatoes the agreement between the obtained data and those calculated on the assumption that the effects of the genes are geometrically cumulative was closer than was the agreement between the obtained data and those calculated on the assumption that the effects of the genes are additive. However, in neither instance was the agreement so close that the differences noted could be attributed to the errors of random sampling. The characters involved were weight of fruits and number of locules per fruit. By using the method involving marker genes, Powers (*36, 37*) has shown that the results obtained in a study of these two characters are those expected if the effects of the genes differentiating these two characters and associated with the marker genes are geometrically cumulative. Hence, it seems rather evident, as previously pointed out by Powers (*37*), from theoretical considerations and a study of other data, that more than one type of interaction of the genes differentiating quantitative characters must exist when measurements of end products are involved.

SUMMARY

The studies here reported included as parental material the Bonny Best, Danmark, and Johannisfeuer varieties of *Lycopersicon esculentum*, and the Red Currant variety of *L. pimpinellifolium*. The breeding was carried to the second hybrid and two backcross generations and in addition the study included the F_1 generation and both parents.

Small size of tomato fruit as determined by weight was found to be partially dominant.

Small number of locules was found to be either completely or partially dominant.

Partial dominance or heterosis was exhibited for smaller number of fruits per unit length of branch.

Heterosis obtained for increased height and spread of plant.

It was concluded that the increase in plant size and the previously determined earliness, due to heterosis (*38*), are sufficient to warrant an intensive study of the possibility of utilizing this phenomenon in the commercial production of tomatoes.

The agreement between the obtained means and those calculated on the assumption that the effects of the genes are geometrically cumulative was closer than was the agreement between the obtained means and those calculated on the assumption that the effects of the genes are arithmetically cumulative. However, in neither instance was the agreement so close that the differences noted could be attributed to the errors of random sampling.

Evidence has been presented showing that from the standpoint of physiological genetics heterosis and dominance are merely different degrees of the same phenomena.

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INHERITANCE OF STAGES OF EARLINESS IN AN INTERSPECIFIC CROSS BETWEEN LYCOPERSICON ESCULENTUM AND L. PIMPINELLIFOLIUM[1]

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INTRODUCTION

In 1936, at the Cheyenne Horticultural Field Station, Cheyenne, Wyo., an interspecific cross was made between the tomato varieties Johannisfeuer (*Lycopersicon esculentum* Mill.) and Red Currant (*L. pimpinellifolium* (Jusl.) Mill.) in an attempt to combine certain important characters of Johannisfeuer with the desirable earliness of Red Currant. The F_1, F_2, and backcross generations, together with the parents, were grown in 1937, and the results on studies of the inheritance of earliness are reported by Powers and Lyon.[3] The F_3 generation and advanced backcross generation, together with the F_1 generation, F_2 generation, and parents, were turned over to the author in 1938 for an investigation of the inheritance of earliness.

METHODS

This study included the parental lines, the F_1 generation, the F_2 generation, F_3 families arising from self-fertilized plants resulting from backcrossing the F_1 generation to Johannisfeuer, and F_3 families arising from self-fertilized F_2 plants. The backcross plants and the F_2 plants giving rise to the last two classes had been selected for earliness of maturity in 1937. The seed was planted in flats in the greenhouse on April 21, 1938. After germination the seedlings were spotted off in flats in strain rows, which were randomized according to Tippett's randomization tables[4] to eliminate possible positional effects in the greenhouse. The strains and generations were transplanted into the field June 1, 1938, in rows, 12 plants to the row, each row being replicated 4 times and randomized as to position within a block. Ten blocks of 48 rows each were used, each block containing both parents, the F_1 and F_2 generations, 4 strains of the selfed generations of the backcross, and 4 strains of selected F_3's. This made possible the testing of 40 strains of the selfed generation of the backcross and 40 strains of F_3's in a randomized block design,[5] using a total population of 5,760 plants.

On July 13, 1938, when the large-scale breeding population was entirely in bloom, a severe hailstorm occurred, inflicting considerable

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damage on the entire population. The effect of the hail was uniform over the entire field planting, and only stubs of plant stems were left above ground. Since hailstorms inflicting such damage occur frequently during this season in many portions of the central Western States, they must be considered as part of the environment in any breeding program. Under conditions that are present in such an environment, inheritable earliness is confused with ability to recover after hail damage.

For convenience and ease of analysis, earliness in tomatoes may be divided into various stages. Powers and Lyon[6] have recognized the following stages and have found they behave as genetic characters and can be treated as such in studies of quantitative inheritance: (1) Period from planting to first bloom; (2) period from first bloom to first fruit set; (3) period from first fruit set to first complete color change. In the experiment herein reported the occurrence of hail delimited additional stages, so that five different stages are employed in this study. The following is a list of the stages, together with the criteria that delimit them in actual field practice:

(1) *Period from planting to first bloom before hail.*—The day that the corolla of the first flower opened was recorded for each plant, and the number of days from the planting date was computed for this stage of earliness.

(2) *Period from planting to first bloom after hail.*— The same procedure was followed as in stage 1, as all plants were pruned back to a uniform basis and all flower buds were removed immediately after damage occurred.

(3) *Period from first bloom after hail to first fruit set.*—When a definite swelling of an ovary in a flower occurred, the date was recorded and the number of days from first bloom was computed for this stage of earliness.

(4) *Period from first fruit set to first complete color change.*— The date of first complete color change was recorded for each plant when the green coloring on a fruit had disappeared, and the number of days from first fruit set to first complete color change was computed for this stage of earliness.

(5) *Period from planting to first complete color change.*- This stage of earliness represents a summation of stages 2, 3, and 4.

The mean values of rows were used to establish differences between strains or generations. The data thus obtained were then subjected to analyses of variance, and the interaction between strains and replications was used as an estimate of error. Odds as great as or greater than 19 : 1 against the deviations noted being due to the errors of random sampling were accepted as being statistically significant.

RESULTS

Table 1 gives the *F* values for testing the statistical significance of differences between strain means and of differences between replication means. The differences between the strain means will be discussed under their respective stage headings.

PERIOD FROM PLANTING TO FIRST BLOOM BEFORE HAIL DAMAGE

From table 1, it may be concluded that, for period 1, in no case could the difference between strain means be accounted for by chance. In all cases the *F* values were of sufficient magnitude to give probability values as small as or less than 0.01.

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[6] See footnote 3.

TABLE 1.—*F values for testing the statistical significance of differences between strain means and of differences between replication means*

BETWEEN STRAINS[1]

Block No.	F value for indicated period: (1) Planting to first bloom (before hail)	(2) Planting to first bloom (after hail)	(3) First bloom after hail to first fruit set	(4) First fruit set to first color change	(5) Planting to first color change
1	10.92	4.23	2.74	1.73	3.20
2	11.45	4.29	1.16	4.85	5.10
3	18.75	6.08	1.67	8.86	13.84
4	7.45	5.83	.37	5.21	9.60
5	5.99	5.58	1.61	3.21	1.92
6	5.54	3.57	1.89	10.46	11.02
7	14.33	6.50	2.53	9.37	11.73
8	3.70	3.07	2.21	2.78	3.65
9	20.85	8.66	1.12	5.11	5.98
10	5.10	5.71	1.39	6.38	10.2[illegible]

BETWEEN REPLICATIONS[2]

Block No.	(1)	(2)	(3)	(4)	(5)
1	0.01	0.26	2.05	3.20	3.19
2	1.[illegible]	2.23	.76	.72	1.11
3	.30	.81	.94	1.68	5.48
4	6.62	1.25	1.00	5.01	6.30
5	.52	2.28	1.18	1.19	1.07
6	2.62	2.36	.80	2.18	3.79
7	.99	3.91	.01	5.45	8.78
8	.79	.24	.90	2.87	1.64
9	.13	1.87	2.20	1[illegible]	[illegible]7
10	.80	1.38	1.60	.40	[illegible]

[1] 2.84 is the 1 percent level and 2.09 is the 5 percent level for values of F with 11 degrees of freedom for strains and 33 degrees of freedom for the interaction of strains and replications, which was used as error.

[2] 4.44 is the 1 percent level and 2.89 is the 5 percent level for values of F with 3 degrees freedom for replications and 33 degrees of freedom for the interaction of strains and replications, which was used as error.

Before hail, the Red Currant parent exhibited a mean number of days to first bloom of 74.55 ± 0.444 days from the planting date. Johannisfeuer, the later parent, bloomed in 81.54 ± 0.271 days, making it possible to demonstrate a parental mean difference of 6.99 ± 0.520 days. The F_1 generation bloomed in 76.13 ± 0.446 days and differed from the arithmetic mean of the two parents (78.04 ± 0.260 days) by 1.91 ± 0.516 days. The mean difference is over three times its standard error and therefore significant. The simple geometric mean (77.97 days) obtained from the means of the parents does not conform to the observed mean of the F_1 generation as a mean difference of 1.84 days is demonstrable. Also, from the foregoing data it is apparent that the F_1 generation differs significantly from the earlier parent, as the difference is 1.58 ± 0.629 days. In other words, although the geometric mean obtained from the means of the two parents more closely approaches the observed mean of the F_1 generation than does the arithmetic mean obtained from the means of the two parents, dominance is a factor and must be considered. This agrees with data of Powers[7] for number of locules and fruit size.

The observed mean of the F_2 generation for date of first bloom before hail was 75.76 ± 0.408 days. By using Wright's formula as quoted by Powers,[7] which takes into consideration dominance in geometric inheritance as shown by the F_1 generation, one may obtain a predicted F_2 mean of 77.04±0.261 days. While the standard error of the predicted F_2 mean is in itself subject to error and represents only the best approximation available, the difference between predicted and observed F_2 means of 1.28±0.484 is statistically significant. Therefore, in considering the F_2 generation, the viewpoint given above of a geometrically inherited character taking into account dominance is not substantiated.

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The data show that it is possible by selection within the F_2 generation to obtain F_3 strains that are at least 8 days earlier than the Red Currant parent in regard to date of first bloom. Table 2 lists 10 such F_3 strains, giving strain means as well as the standard errors.

TABLE 2.—*Tabulation of the mean number of days from planting to first bloom before hail of parents, F_1 and F_2 generations, and early selected F_3 strains*

Parent or strain No.	Mean	Error	Generation or strain No.	Mean	Error
	Days	*Days*		*Days*	*Days*
Parent			Generation		
Red Currant	74.55	±0.444	F_1	76.13	±0.446
Johannisfeuer	81.54	±.271	F_2	75.76	±.408
Selected F_3 strain			Selected F_3 strain[1]		
26-10	66.02	±3.830	98-12	62.50	±2.423
40-7	57.25	±1.691	144-3	61.31	±2.188
60-15	56.20	±3.529	210-8	60.87	±1.119
72-23	66.13	±1.425	211-4	64.19	±3.309
97-16	63.04	±3.589	216-1	66.44	±1.192

[1] These strains were at least 8 days earlier than the Red Currant parent in the period from planting to first bloom before hail.

PERIOD FROM PLANTING TO FIRST BLOOM AFTER HAIL DAMAGE

From table 1 it is clearly demonstrable that any differences shown between strain means are valid and significant. In all of the 10 blocks, one is able to demonstrate significance above the 1-percent point for the differences between means of the strains, or, in all cases the odds are greater than 99:1 against the occurrence by chance of such a distribution of strain means.

After the hailstorm the Red Currant parent bloomed in 98.26±0.195 days from the date of planting. The later parent, Johannisfeuer, bloomed in 102.02±0.189 days, and it is possible after hail damage to demonstrate a mean difference between parental lines of only 3.76 ±0.272 days in blooming dates. When one recalls the difference of 6.99±0.520 days that was demonstrable before hail occured, one may conclude that the expression of this genetic difference was decreased, in this experiment, by damage due to hail.

The F_1 generation shows a mean number of days to first bloom of 97.89±0.209. This mean conforms neither to the arithmetic mean (100.14±0.136 days) obtained from the means of parental lines nor to the simple geometric mean (100.12 days) obtained in the same manner. It is impossible to demonstrate conclusively that the F_1 generation is significantly earlier than the Red Currant parent, since a difference of only 0.37±0.286 day is shown.

The observed F_2 mean was 99.13±0.194 days, which is very close to the predicted 99.00±0.125 days based on the assumption that genes differentiating this stage of development are geometrically cumulative. Refined analysis is not necessary to show that the difference between these two means is not statistically significant.

The selected F_3 strains that had shown earliness in regard to date of first bloom before hail gave no indications whatsoever of blooming early after hail damage occurred. There was no indication of early blooming after hail in any F_3 strain or in any strain of the selfed generation of the backcross, and the F_1 generation in general showed the only indications in the population of blooming earlier than the Red Currant parent.

PERIOD FROM FIRST BLOOM TO FIRST FRUIT SET

The Red Currant variety of tomato requires a period from first bloom to first fruit set of 4.25±0.090 days, while the Johannisfeuer variety requires a shorter period of 3.58±0.121 days. The parental strains thus exhibit a significant mean difference of 0.67±0.151 day. The F_1 generation mean (3.88±0.109 days) lies between those of the parents and differs significantly from the mean of the Red Currant parent, the mean difference being 0.37±0.141 day. The difference between the Johannisfeuer parent and the F_1 generation (0.30±0.163 day) closely approaches statistical significance. There is, however, no significant difference demonstrable between the F_1 generation and the arithmetic mean (3.91±0.075 days) calculated from the means of the parents or between the F_1 generation and the geometric mean (3.90 days) calculated from the means of the parents.

The F_2 generation, with a mean of 4.05±0.094 days for this stage of earliness, conforms to both the arithmetic and geometric means calculated from the means of the two parents, as no significant differences are involved. It also conforms to the predicted F_2 value (3.89±0.067 days) considering dominance of the heterozygote, although in this case the F_1 generation mean lies between the means of the two parents.

By a comparison of any two strain means, it is not possible to demonstrate that statistically significant differences between strains exist within any particular block. (See table 1.) However, directional effects between genotypes are constant within blocks; and because of the large population involved, with 40 replications within parents and within generations, errors are reduced to a minimum. Accepting, then, the errors obtained by the analysis of variance within parents and generations, it is possible to demonstrate a small but significant difference between inbred lines. However, the differences exhibited in this stage of earliness in this particular cross in the year 1938 were so small as to be negligible in a practical consideration of earliness in general. In other years, under different environmental conditions, when the character would include another factor, blossom drop, it might prove to be highly important.

PERIOD FROM FIRST FRUIT SET TO FIRST COMPLETE COLOR CHANGE

From table 1, it can be seen that the *F* values for testing significance of mean differences for the stage from first fruit set to first complete color change are statistically significant for 9 of the 10 blocks. A mean of 49.81±0.480 days was obtained for the Red Currant variety of tomato, while the calculated mean for the Johannisfeuer variety was 52.09±0.397 days. A parental mean difference of 2.28±0.623 days is thus shown. The F_1 generation shows heterosis, with a generation mean of 46.07±0.520 days, and clearly can conform in no way to either the arithmetic mean (50.95±0.311 days) or the geometric mean (50.94 days) obtained from the means of the parents.

The F_2 mean of 47.76±0.430 days conforms closely to the predicted mean of 48.44±0.311 days obtained by use of Wright's formula. The difference between means of 0.68±0.531 day is not statistically significant. It is possible by selection within the F_2 generation to obtain F_3 strains that are at least 6 days earlier than the Red Currant parent in the period from first fruit set to first complete color change. Table 3 lists 10 such selected F_3 strains, together with their standard errors.

TABLE 3.—*Tabulation, in mean number of days, for the period from first fruit set to first complete color change of the parents, the F_1 and F_2 generations, and the early selected F_3 strains*

Parent or strain No	Mean	Error	Generation or strain No	Mean	
Parent	*Days*	*Days*	Generation	*Days*	*Days*
Red Currant	49.81	±0.480	F_1	46.07	±0.520
Johannisfeuer	52.00	±.397	F	47.76	±.430
Selected F_3 strain			Selected F_3 strain [1]		
60-15	42.81	±1.758	134-1	44.17	±1.782
65-8	42.02	±.995	144-3	42.02	±.405
104-2	42.91	±1.580	173-10	42.58	±1.750
106-15	40.38	±1.035	205-2	42.94	±.821
115-1	40.56	±.410	211-1	41.12	±.761

[1] These strains were at least 6 days earlier than the Red Currant parent in the period from first fruit set to first complete color change

PERIOD FROM PLANTING TO FIRST COMPLETE COLOR CHANGE

As one would expect from table 1, if significance is obtained for "between strains" in any of the various stages of earliness and no compensating directional effect between stages is in evidence, significance must of necessity be present in the summation of all stages from planting to first complete color change. It will be noted in stage 5 (see table 1) that the odds are greater than 99: 1 in all blocks against the occurrence by chance of such a distribution of strain means. Table 4 represents a summation of all the data on stages of earliness for the parents, the F_1 generation, and the F_2 generation.

It is shown in all cases except stage 3, where only minute differences are demonstrable, that the differences between parents are all in the same direction. The final mean difference exhibited between the inbred lines is 5.43±0.654 days and is less than the mean difference shown in the same parents up to the date of first bloom before hail damage occurred. In years when injury does not occur,[8] greater differences between parents may be expected than are noted here.

The F_1 generation in stage 5 shows marked heterosis in earliness as a result of summation of effects shown in stages 2 and 4. The mean of the F_2 generation lies between the means of the F_1 generation and the earlier parent, as it did in stage 4, where heterosis in the earliness of the F_1 generation was also noted. The mean of the F_2 generation is clearly predictable, as it is in all stages of earliness after hail damage occurred, in which the calculations are based on the assumptions that the effects of the genes are geometrically cumulative and that dominance as exhibited by the F_1 generation must be taken into account.

The F_3 strains that showed earliness when the period from planting to first complete color change was used as a criterion were those strains that exhibited earliness in a particular stage. In some cases earliness in one stage was compensated by lateness in another stage, and the result was a strain of no value as far as earliness in general is concerned.

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[8] See footnote 3

It is of interest to examine the results of the analyses of variance for "between replications." Whether position in the field materially affects these stages of earliness may be determined from the corresponding F values. In 11 cases the F values (see table 1) are of sufficient magnitude to give P values as low as or lower than 0.05. In the character, period from first fruit set to first complete color change, four blocks showed significant F values and, in all cases, this was reflected in the same blocks by significance for "between replications" in the summation of all stages from planting to first complete color change.

TABLE 4.—*Complete analysis of earliness, showing generation means in days with their standard errors for the various stages of earliness*

Stage of earliness	Red Currant parent	Johannisfeuer parent	Mean difference between parents	F_1 generation	F_2 generation	Mean difference between actual F_2 means and predicted F_2 means according to Wright's formula
	Days	*Days*	*Days*	*Days*	*Days*	*Days*
(1) Planting to first bloom before hail[1]	71.55±0.144	81.51±0.271	6.98±0.320	76.13±0.146	75.76±0.108	1.29±0.184
(2) Planting to first bloom after hail	98.26±0.195	102.02±0.189	3.76±0.272	97.89±0.209	99.13±0.194	1.31±0.231
(3) First bloom to first fruit set	4.27±0.090	3.58±0.121	0.68±0.151	3.88±0.109	4.05±0.094	0.16±0.115
(4) First fruit set to first color change	49.81±0.180	52.09±0.397	2.28±0.623	[illegible].07±0.520	47.76±0.430	0.68±0.531
(5) Date planted to date of first color change	152.17±0.509	157.60±0.410	5.43±0.651	147.69±0.528	150.81±0.473	

[1] Severe hail damage occurred at this particular time leaving only plant stems above ground. All subsequent stages of earliness are confused with recovery effects.

Since in 11 cases the F values for "between replications" were statistically significant, it is apparent that stages of earliness are affected by the environmental influences represented by position in the greenhouse or field, or both. Further, from the fact that a majority of the statistically significant F values of the replications fall within the period from first fruit set to first color change and the entire time from planting to first color change, one may conclude that as regards this cross the stage from first fruit set to first color change is more affected by position in the field than are the other stages of earliness.

SUMMARY

A population of 5,760 plants of a cross between *Lycopersicon esculentum* Mill. and *L. pimpinellifolium* (Jusl.) Mill., which included both parents, the F_1 generation, and the advanced generations, was grown at Cheyenne, Wyo., in 1938. Earliness was broken up into its constituent stages, and the number of days for each stage was recorded for each plant.

Before hail damage the parents showed a difference of 7 days between means in the period from planting to the date of first bloom.

The mean of the F_1 generation was significantly higher than that of the earlier parent and differed significantly from both arithmetic and geometric means of the parents. The actual F_2 mean did not conform to the predicted F_2 mean based on the assumptions that the effects of the genes are geometrically cumulative and that dominance as exhibited by the F_1 generation must be taken into account. It is possible, by selection of F_2 plants, to obtain F_3 strains that are earlier than either parent in this stage of earliness.

After hail damage, there was a difference of only 4 days between the means of the parents in date of first bloom. The F_3 strains that showed earliness in date of first bloom before hail showed no indications of earliness in date of first bloom after hail. The period from planting of seed to date of first bloom after hail was found to be shorter for the F_1 generation than for either of the parents or for any other generation. The F_2 mean is clearly predictable in this stage of earliness if one assumes that the effects of the genes are geometrically cumulative and that dominance as exhibited by the F_1 generation must be taken into account.

Only minute differences are demonstrable in the period from first bloom to first fruit set after hail, and the differences are found to be so small as to be negligible in a practical breeding program.

Significant differences are demonstrable between the parents in the period from first fruit set to first complete color change. The F_1 generation shows heterosis in this stage of earliness, and the F_2 generation mean is predictable if one assumes cumulative gene effects, taking into account dominance as shown by the F_1 generation. It is possible by selection to obtain F_3 strains that are earlier than either parent in this stage of earliness.

Any differences noted in earliness, when number of days from planting of the seed to first complete color change is the criterion, are merely the result of a summation of stage differences as regards the present study.

THE BUFFER CAPACITY OF THE BLOOD OF THE SIXTH-INSTAR SOUTHERN ARMYWORM (PRODENIA ERIDANIA)[1]

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INTRODUCTION

It is well known that biochemical reactions take place in buffered media and that minor changes in hydrogen-ion concentration can affect profoundly the course of reactions. In the blood of man a variation of more than ±0.1 pH from the normal 7.4 is considered pathological, and a change greater than ±0.4 pH is usually fatal. The pH of the blood is maintained within these narrow limits by a system of buffers whose action is well understood. The buffer effect of the various constituents of the blood, as well as its buffer capacity under both normal and pathological conditions, has been studied by Peters and Van Slyke (*14*)[2] and others.

The blood of several marine invertebrates has also been found to be well buffered. Redfield et al. (*16*) summarized the early work in this field and showed that hemocyanin was the main buffer in the blood of *Limulus polyphemus*.

The buffer capacity of the blood of only a few insects seems to have been investigated, but the blood of those studied is well buffered.

The limits within which the pH of insect bood can range without resulting in the death of the insect appear not to have been determined. The variation from species to species and between individuals of the same species, however, is rather large. The pH of the blood of several species, as determined by Kocian and Špaček (*10*), Bodine (*4*), Ludwig (*11*), Babers (*1*), and others, varied between 6.0 and 7.3. Kocian and Špaček found that in the same species the blood from individual insects might vary as much as 0.4 pH.

The blood of *Dytiscus marginalis* L. was alkaline to litmus, according to Barratt and Arnold (*2*), and remained so when an equal volume of 0.03 N hydrochloric acid was added, but it became acid when an equal volume of 0.1 N acid was used. The same authors found the blood of *Hydrophilus piceus* (L.) to be alkaline to litmus but neutral when an equal volume of 0.025 N acid was added. Craig and Clark (*7*) titrated the blood of *Pieris rapae* L. and *Heliothis armigera* (Hbn.) (*H. obsoleta* (F.))[3] with 0.1 N acid and alkali and determined the buffer capacity of the two bloods. These authors give the pH of the blood of *P. rapae* larvae as 7.17. This value is of interest because of the relative scarcity of reports of alkaline insect blood, and also because

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[1] Received for publication March 5, 1941

[2] Italic numbers in parentheses refer to Literature Cited, p. 189.

[3] *Heliothis obsoleta* was used in the reference cited, whereas the name currently in use is *H. armigera* (Hbn.)

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Fink (*9*), using colorimetric methods, found the blood of this larva to vary between pH 6.2 and 6.4. Using the glass electrode, the present writer has found (unpublished results) the average pH of the blood of this insect to be 6.52. Brecher (*5*) found the blood of *P. brassicae* (L.) to be well buffered. She attributed the buffering power to phosphates, which she found to be present in 0.02-molar concentration. Duval and Portier (*8*), using a number of species, found the hydrogen-ion concentration of the blood to be very stable. They also attributed the buffering power to the presence of phosphates. The blood of *Bombyx mori* L. had an acidity equivalent to 0.46 gm. of oxalic acid per 100 cc. when Nazari (*13*) titrated samples to phenolphthalein with 0.1 N potassium hydroxide.

At the present time large numbers of the southern armyworm (*Prodenia eridania* (Cram.)) are being used in various physiological and toxicological investigations. A knowledge of the buffer capacity of the blood of the larvae of this insect and the effect of nicotine vapor on its blood pH has become desirable. The present report is the result of such a study.

MATERIALS AND METHODS

Mature, actively feeding sixth instars that had been reared in greenhouse cages on turnip and collard foliage were used. Blood was collected by snipping a proleg while it was held under mineral oil. By this treatment loss of carbon dioxide was held at a minimum, and the darkening of the blood that occurs on exposure to air was greatly retarded. When unfixed blood was used in this manner the cells coagulated rapidly, but it was thought that more drastic changes might occur if heat-fixed blood were used. Hydrogen-ion determinations were made on pooled samples of definite volume by the use of a small, ordinary bulb-type glass electrode in connection with a saturated calomel half-cell. The pH was read directly from a calibrated potentiometer dial after a zero reading had been obtained by the use of a standard buffer; the dial could be read to about ±0.02 pH. Small increments of acid or alkali were added from a microburette, and the resulting pH was read. Titration curves were plotted in which the molar concentration of acid or alkali was the ordinate and the corresponding pH the abscissa.

The buffer capacity, β, is defined by Van Slyke (*17*) as the "differential ratio dB/dpH, expressing the relation between the increment (in gram-equivalents per liter) of strong base B added to a buffer solution and the resultant increment in pH. Increment of strong acid is equivalent to a negative increment of base, or $-$dB." Since the increment of pH obtained when acid is added is also negative, the value β is always positive. If sufficiently small increments of alkali are used, β can be calculated directly. However, since β is equal to the tangent of the titration curve at the corresponding pH, it is usually more convenient to obtain it in this manner, and this method was used in the determinations. Theoretical discussions of buffers and buffer capacities are given by Van Slyke (*17*), Peters and Van Slyke (*14*), and Clark (*6*).

The carbon dioxide content was determined both on pooled samples and on the blood of individual larvae by the manometric method of Van Slyke and Neill (*18*) as described by Peters and Van Slyke (*15*).

RESULTS

The pH of the blood as drawn under oil varied between 6.50 and 6.75, with an average of 6.65. A composite titration curve from the several experiments is shown in figure 1. The buffer-capacity curve in figure 2 was obtained by plotting the several values of β obtained from the titration curve against the corresponding pH value. The total carbon dioxide content of the blood as drawn varied between 8.40 and 13.45 volumes percent, with a mean of 10.03, for 10 analyses, or about 4.51 millimoles per liter.

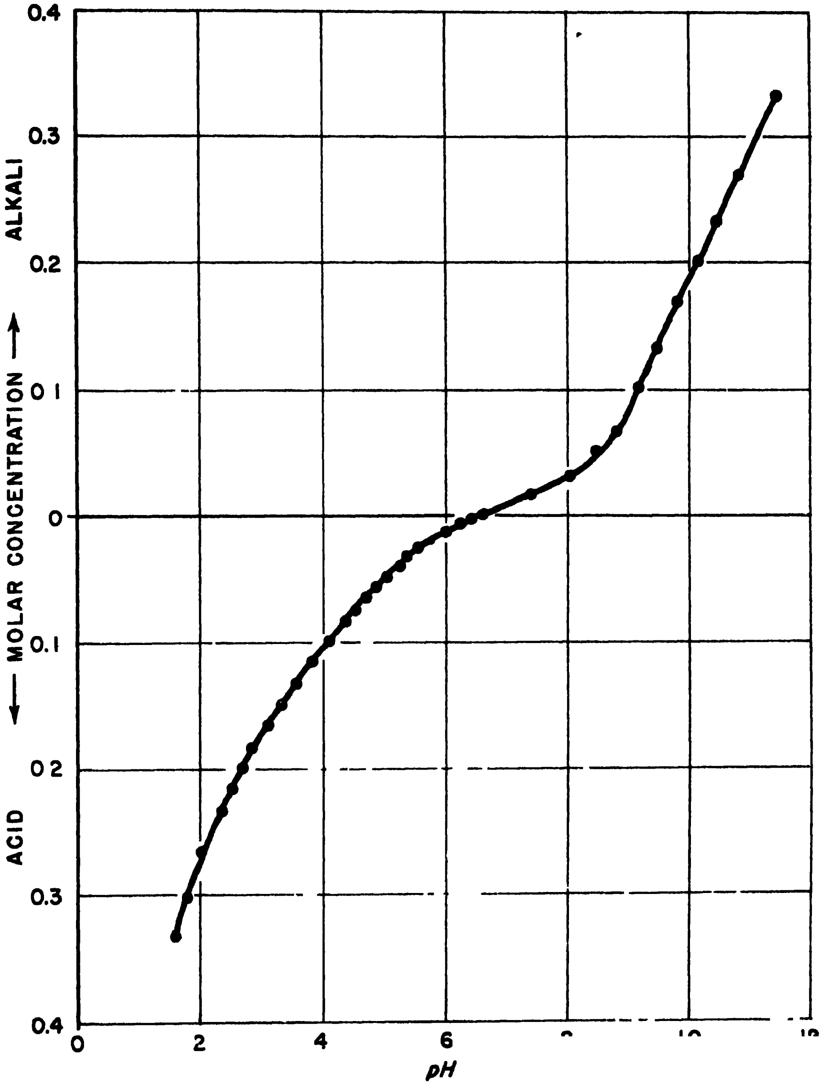

FIGURE 1. Titration curve for the hemolymph of sixth-instar southern armyworms.

Bishop (*3*) has found the dissociation constant, pK′, for the blood of honeybee larva to be 6.1, the same value as that given by Peters and Van Slyke for human serum. If this value holds for the blood of the southern armyworm, the ratio of bicarbonate to carbonate as obtained from the Henderson-Hasselbalch (*14*) equation

$$\text{(1)} \qquad pH = pK' + \log \frac{[BHCO_3]}{[H_2CO_3]}$$

ranges from 3.55 to 1.

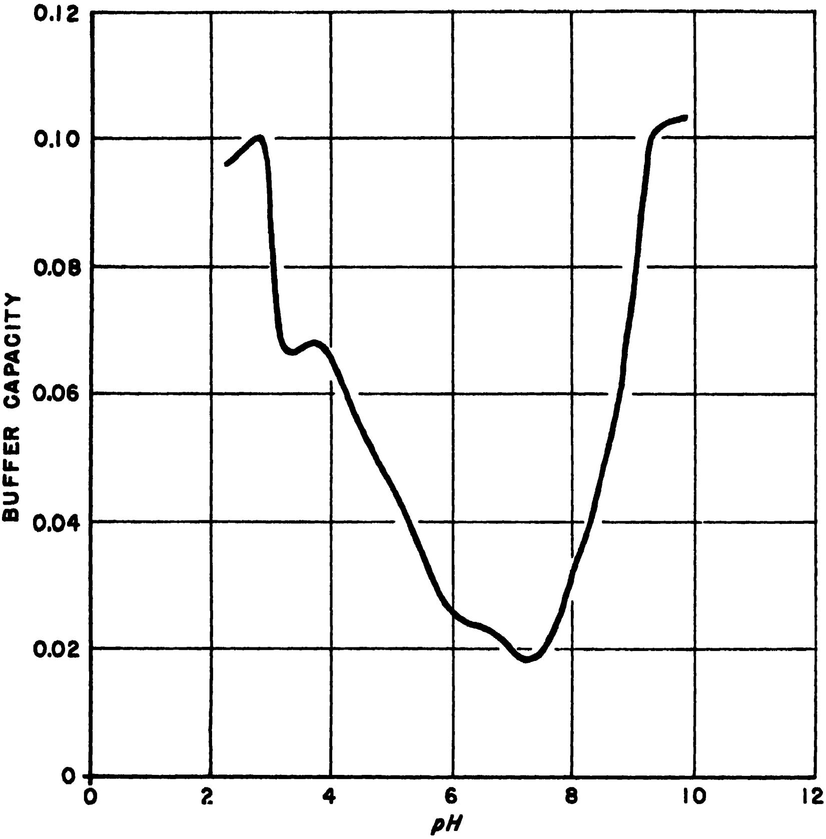

FIGURE 2. Buffer capacity of the hemolymph of sixth-instar southern armyworms

The solubility coefficient, α, of carbon dioxide in human serum at 38° C. is 0.92 times that of water. If the same ratio holds for larval blood, α at 25° will be 0.695. From the Henderson-Hasselbalch equation in the forms

$$\text{(2)} \qquad mM[H_2CO_3] = 0.0591\alpha p$$

$$\text{(3)} \qquad mM[BHCO_3] = [CO_2] - 0.0591\alpha p$$

$$\text{(4)} \qquad p = \frac{[CO_2]}{0.0591\alpha(10^{pH-pK'}+1)}$$

the carbonic acid content of the larval blood as drawn is 0.99 millimole per liter, the bicarbonate content is 3.52 millimoles per liter, and the carbon dioxide tension (*p*) is 24.2 mm. of mercury.

The late instars of the southern armyworm have been found by Richardson[4] to be very resistant to nicotine. Sixth instars were therefore kept for 24 hours at room temperature in an atmosphere saturated with nicotine. The larvae, still alive although motionless, were then carefully washed with distilled water, the blood was removed in the usual manner, and its pH determined. Although nicotine is a strong base, having a dissociation constant of 9×10^{-7}, the pH of the blood was still its normal 6.65. Efforts were made to determine the amount of nicotine in the blood, but they were not entirely successful. The blood was made slightly alkaline with sodium hydroxide and distilled. The distillate had a distinct odor of nicotine, but the color obtained after treatment with Markwood's (*12*) reagents could not be matched directly with that of a nicotine standard. Since ammonia interferes with this test, the difficulty was probably due to the presence of ammonia, which was formed during the distillation from the urea and uric acid normally present in the blood. A match in colors was finally obtained by use of a Wratten 8 No. 58 filter, but too much faith cannot be placed in the quantitative nature of the result. By this method the blood contained 7.15 mg. of nicotine alkaloid per 100 ml.

DISCUSSION

Since most metabolic waste products of animal origin are acid, one would expect the blood to be buffered mainly on the acid side. In the bloods that have been studied this has been found to be true.

The buffering power of human blood is due mainly to the alkaline salts of the proteins, hemoglobin being the most efficient, and to the bicarbonates. For the neutralization of volatile acids such as carbonic, hemoglobin is the most efficient because of its buffering action. For the neutralization of nonvolatile acids such as β-hydroxybutyric the bicarbonates are effective, not because of a true buffer action, but because of the efficiency of the lungs in removing the carbon dioxide that is liberated.

Since the buffer capacity is dependent on the concentration of the buffer material, the inorganic phosphates play only a minor role in the buffer system of human blood. Only 3 to 4 mg. of inorganic phosphorus is present per 100 ml. of blood, corresponding to about a 0.001-molar solution, and it has been found that normally blood can neutralize about 28 ml. of normal acid per liter within the physiological range.

At its average normal pH of 6.65 the blood of the southern armyworm has a buffer capacity of 0.022, which is approximately equivalent to a 0.038-molar solution and compares with the buffer capacity of 0.0228 for human blood at pH 7.4, as reported by Van Slyke (*17*).

In the insects whose buffer systems have been studied hemoglobin and hemocyanin do not occur; the buffering power of the blood is therefore due to an entirely different system from that of man and the marine invertebrates. The carbon dioxide content of human blood is between 45 and 60 volumes percent, about 95 percent of which is combined as bicarbonate. In insects the total carbon dioxide content is much lower, the blood of the southern armyworm containing only about 10 volumes percent, which corresponds to approximately 4.5 millimoles per liter, 3.52 millimoles, or 78 percent, of which is in the form of bicarbonate. Because of the small amount present it seems unlikely that bicarbonates are as active as buffers as they are in the higher animals. They do probably play some part, however. In the honeybee, where the average carbon dioxide content of the blood was found by Bishop (*3*) to be 29.2 volumes percent, the bicarbonates may play a more important role.

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[4] Unpublished manuscript

That compounds other than the salts of the weak acids are responsible for a considerable part of the buffering power of the blood of the insect is apparent from a consideration of the figures obtained by chemical analysis (*1*). Of the acids whose salts might act as buffers, inorganic phosphorus was 0.0056 molar, uric acid 0.00088 molar, and bicarbonates 0.00352 molar, a total equal to about a 0.01-molar solution. It must be noted, however, that the most effective buffer range of the salts of these acids is very near the normal pH of the insect blood. Van Slyke (*17*) has shown that the maximum buffer effect is exerted when $pH = pK'$. For the phosphates, urates, and bicarbonates these points are 6.8, 5.64, and 6.1, respectively.

It has been shown (*14, p. 892*), however, that, in addition to the inorganic phosphates, certain phosphorus-containing organic compounds also act as buffers in human blood. It is therefore probable that at least part of the organic phosphorus in armyworm blood is likewise available. This would, of course, reduce the buffering power unaccounted for, because the larval blood contains about 105 mg. of organic phosphorus per 100 ml., or about 0.034 mole per liter.

In human blood the content of the amino acids is so low that their slight buffering effect is exerted far from the neutral point. In insect blood, however, the amino acids are present in large quantities; the southern armyworm larval blood, for instance, contains 2.352 gm. of amino nitrogen per liter, which corresponds to about a 0.16-molar solution of amino acids. It is probable, therefore, that salts of amino acids and salts of proteins also play a considerable part in the buffering system of the insect's blood.

It is probable that in insects diffusion will account for both the intake of oxygen and the elimination of carbon dioxide. The insect possesses a circulatory system by which the hemolymph is kept in circulation, but so far as is known no experiments have been made that would show a difference in composition at any stage of the circulatory process. In man the reaction of the blood is dependent on the $BHCO_3/H_2CO_3$ ratio, which in turn is determined by the ratio of carbon dioxide production to elimination, while the $BHCO_3$ concentration depends on the amount of alkali in the body that is not combined with acids other than carbonic. The alkalinity of the blood as shown by the pH is a measure of the efficiency of the system, a low pH being direct evidence of the impairment of efficiency. It also seems probable that the low pH of insects' blood, being usually far on the acid side, is due, in some part at least, to inefficiency in the carbon dioxide excretory mechanism.

SUMMARY

The buffer capacity of the blood of sixth instars of the southern armyworm (*Prodenia eridania* (Cram.)) has been determined from titration curves. This value at pH 6.65, the normal for the blood, is 0.022.

The total carbon dioxide content of freshly drawn blood is 10.03 volumes percent, corresponding to 4.51 millimoles per liter. Of this, 3.52 millimoles is combined as bicarbonate and 0.99 millimole as carbonic acid.

Blood from larvae kept in an atmosphere saturated with nicotine for 24 hours did not change in pH, although the blood had absorbed considerable nicotine.

The buffering power of the blood is probably due to the presence of bicarbonates, phosphates, urates, and the salts of the proteins. The phosphorus-containing organic compounds and the amino acids also probably play an important role in the buffer system.

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SURVIVAL AND DEVELOPMENT AT LOW TEMPERATURES OF EGGS AND PREINFECTIVE LARVAE OF HORSE STRONGYLES[1]

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INTRODUCTION

The preparasitic, or free-living phase, of the life cycle of the strongylid nematodes parasitic in horses includes the egg, the first- and second-stage preinfective larvae, and the ensheathed or infective larva. Among strongyles generally, these stages are vulnerable to the effects of environmental factors. Temperature, moisture, and sunlight are generally considered to be the most important natural influences affecting the survival and development of nematode eggs and larvae.

Studies in which the effects of but a single factor of the environmental complex are investigated either in laboratory or outd or experiments have been criticized as academic and of doubtful practical significance, since in nature the various elements of the complex ordinarily function simultaneously as determinants of the survival and development of eggs and larvae. Field studies in which the effect of the environment as a whole may be determined are undoubtedly of great value. However, the results of such studies have validity under identical or very similar conditions only. Even in the same locality meteorological conditions rarely or never are duplicated from year to year. Moreover, by themselves, such studies cannot demonstrate which factor or factors in the complex caused the observed result or the degree to which each participated in the result. Hence, both types of investigation apparently have their limitations, and the formulation of generalizations, interpretations, and expressions of probability, so far as that is possible and advisable, should be based on the information elicited by investigations of both types.

In all experiments reported in this paper, desiccation, excess moisture, and direct sunlight were eliminated or minimized as causative factors and, consequently, temperature was the principal element in the causation of the observed experimental results.

The specific objectives of the investigation were to determine (1) the resistance of the eggs and preinfective larvae of horse strongyles to subfreezing temperatures; (2) the survival time of these stages at a mean temperature of 36° F., which presumably would inhibit appreciable development; and (3) the survival and development of these stages in feces exposed to temperatures prevailing during winter and early spring in the vicinity of the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., where all the experiments were conducted in 1938 and 1939.

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The resistance of infective horse strongyle larvae to cold was not studied since, as a result of work by other investigators, it appears to be well established that these larvae can endure very low winter temperatures. The observations on infective larvae that are included in this paper are, therefore, merely incidental to the principal objectives. The effects of cold on the eggs and preinfective larvae also have been investigated by others, but definite information on this subject appears to be lacking.

REVIEW OF LITERATURE

Albrecht (*1*)[2] stated that cold delays the development of horse strongyle eggs but does not exert an injurious effect on them. Hueber (*7*) reported that the eggs withstand cold for a long time. De Blieck and Baudet (*4*) found that cold hinders development of the eggs. They observed that eggs kept for 12 days at 6° C. did not hatch, but many contained living larvae. According to these authors, eggs in the morula stage kept at 0° C. for 12 days did not undergo development but retained their vitality. However, when exposed to temperatures ranging from —15° to —20° for 6 hours, all eggs were killed. Gackstatter (*6*) reported that hard freezing for 15 days of eggs in fecal balls, or for 10 days in feces that had been broken up, did not reduce the vitality of the eggs but merely arrested their development. This author also noted that eggs kept for 10 days at 2° to 3° C. were still capable of development.

According to Enigk (*5*), uterine eggs on agar cultures were not all killed at —8° C. in 45 days, but none developed after 56 days at this temperature. Enigk reported, however, that fresh fecal pellets kept at —8° for 55 days yielded several colonies of larvae when the feces were cultured. A few small colonies also developed in cultures consisting of pellets that had been broken up and exposed at this temperature for 41 days. Wetzel (*13*) stated that a rather high percentage of eggs of *Strongylus equinus* tolerated freezing temperatures near 0° C. for several hours.

Parnell (*9*) stated that horse strongyle eggs, before embryonation, are very resistant to the extremes of temperature and climate encountered in Canada. He reported that the continual low temperatures of a severe Canadian winter were insufficient to kill "sclerostome" eggs, that these temperatures merely inhibited development, and that the eggs embryonated and hatched normally in the spring. He also stated that embryonated eggs are much less resistant and are relatively easily killed. He gave no experimental basis for these statements but later (*10*) described an experiment in which feces containing eggs and feces containing other free-living stages were exposed to winter temperatures on Montreal Island for 3½ months, apparently from December 3, 1934, to April 1, 1935. The average mean temperature in that vicinity is given as —15° C. in January; daily fluctuations in temperature may be considerable. Cultures prepared in the spring from the feces that had originally contained unembryonated eggs yielded infective larvae. The experiment was interpreted in part as indicating the great resistance of the eggs to cold.

Baker, Salisbury, and Britton (*2*) reported recently that in an experiment in the vicinity of Ithaca, N. Y., overwintering of horse strongyle eggs on pasture was apparently lethal to 68 percent of these eggs in manure deposited normally and to 100 percent of the eggs in feces spread in a thin layer.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 217.

Observations on the survival of preinfective horse strongyle larvae at low temperatures are scant. De Blieck and Baudet (*4*) found that all newly hatched rhabditiform larvae placed in water and exposed at −15° to −20° C. were killed in 6 hours. Wetzel (*13*) stated that a rather high percentage of preinfective larvae of *Strongylus equinus* tolerated freezing temperatures near 0° C. for several hours. Parnell (*9*) stated that the free-feeding larval stages are much less resistant than the eggs and are relatively easily killed. Later, on the basis of the experiment already mentioned, he (*10*) repeated this statement.

Parnell (*9*) also stated that frequent alternate freezing and thawing can be relied on to kill the free-living stages of horse strongyles. His previously mentioned experiment (*10*) was interpreted as indicating the susceptibility of all free-living stages of these parasites to violent and frequent fluctuations in temperature. Similar but somewhat more specific views were expressed by Britton (*3*) in a summary of the work of several investigators on the factors affecting the viability and development of free-living stages of horse strongyles. He stated that "alternate freezing and thawing can be relied upon to kill all preparasitic stages providing the thaw does not last longer than two weeks," and "during a thaw many eggs which are lying dormant on the field will hatch and the preinfective stages develop slowly and if the thaw is followed by freezing temperatures these forms will be killed as they are susceptible to these temperatures." Examination of the papers enumerated by Britton in connection with his summary has failed to reveal the exact counterpart of these statements. They represent in part his interpretation of the literature that he reviewed.

Wetzel (*13*) stated that an analysis of temperature data for a 30-year period showed that at Hanover, Germany, development of infective larvae of *Strongylus equinus* in the open seems possible from about the middle of March until the middle of November in a normal year.

To summarize, it has been shown in laboratory experiments that horse strongyle eggs are moderately resistant to subfreezing temperatures, but the degree of their resistance has not been defined precisely. The results of one of the two reported outdoor experiments on the effects of cold on the eggs appear to be inconsistent with the laboratory findings. Precise information as to the ability of the preinfective larvae to withstand freezing is lacking. It is known that these larvae are less resistant than the eggs, but the available evidence does not permit a more exact comparison. The ability of eggs and preinfective larvae to survive at temperatures slightly above the freezing point scarcely has been explored. The literature contains statements as to the susceptibility of all free-living stages to alternate freezing and thawing and to violent fluctuations in winter temperatures, but, in the writer's opinion, these statements rest on inference rather than on conclusive experimental evidence so far as the effects of these changes on eggs and preinfective larvae are concerned.

METHODS AND PROCEDURE

The feces used in this investigation, which included both laboratory and outdoor experiments, contained the free-living stages of species of *Strongylus* and of the subfamily Cyathostominae, the latter predominating. It was not determined whether the free-living stages of these two closely related systematic groups react differently to low temperatures. The experimental results apply to the free-living stages of horse strongyles in general.

The feces used in all experiments were collected 2 hours or less after being passed. Feces collected from several horses were used in the investigation. However, only feces from the same collection and from one horse were used for the cultures of an individual experiment. In some cases feces of a single collection from one horse were used in more than one experiment. Immediately after collection, the fecal pellets were broken up, and weighed portions of the well-mixed fecal mass were placed in wide-mouthed glass bottles or jars

In all experiments involving the exposure of eggs, the bottles containing the feces to serve as control cultures were immediately covered with glass lids, and after they had been kept for a week or more at room temperature (65°-85° F.), the feces were examined for infective larvae.

In the laboratory experiments the following procedure was used: To test the resistance of unembryonated eggs to subfreezing temperatures, the test bottles were corked, as a rule, and immediately placed in the freezing compartment of an electric household refrigerator. Exceptionally, glass lids were used to cover bottles during exposure. Following the desired exposure, the bottles were removed from the freezer and kept at room temperature for a week or more before the feces were examined for infective larvae. If the bottles had been corked during exposure, glass lids were substituted for the corks when the bottles were removed from the freezer.

To test the effect of embryonation on the resistance of the eggs to subfreezing temperatures, the procedure was essentially the same as in the experiments with the unembryonated eggs. For purposes of comparison, bottles of one series were immediately corked and placed in the freezer and bottles of a second series were covered with glass lids and kept at room temperature long enough to permit the eggs to embryonate. They were then corked and placed in the freezer.

To test the effect, on the eggs, of continuous exposure to a mean temperature of about 36° F., the test bottles were covered with glass lids and placed in a large cooler, the temperature of which was regulated with a thermostat. From time to time, as was necessary, water was added to each bottle to keep the feces moist. When, after exposure, the bottles were removed from the cooler they were kept at room temperature for at least a week before the feces were examined for infective larvae.

The procedures involved in determining the effects of repeated freezing and thawing were somewhat complicated and consequently are best shown in connection with the results. However, as in the procedures already described, the test bottles were corked while in the freezer but were covered with glass lids when they were removed for exposure to room temperature.

In experiments in which feces containing unembryonated eggs were placed outdoors to observe the effects of winter temperatures on the free-living stages, the test bottles were covered with glass lids at all times. The number of eggs per gram of the fresh mixed feces used for the preparation of cultures was determined. At intervals two or three of the test bottles were brought indoors after identical exposure. When the bottles were brought into the laboratory, usually the egg count per gram of exposed feces, and invariably the number of larvae per culture, were determined. One bottle was kept at room temperature at least 1 week, and the feces were then examined for infective larvae.

In both the laboratory and outdoor experiments to test the effects of cold on preinfective larvae, the bottles containing the feces were covered with glass lids and kept at room temperature long enough to permit the desired stage to develop. The test cultures were then exposed, and the control cultures were immediately examined for larvae unless otherwise indicated in the tables. For exposure of preinfective larvae to subfreezing temperatures or to a mean temperature of about 36° F., other procedure was the same as in the respective experiments with eggs, except that the test cultures were examined only for surviving larvae immediately on removal from the freezer or cooler, unless otherwise indicated in the tables. At various intervals during the outdoor experiment, two identically exposed cultures usually were brought into the laboratory at a time. One was examined immediately for larvae and the other for infective larvae after it had been kept at room temperature for about a week.

To determine the temperatures to which feces were exposed in the freezing experiments, a maximum and minimum recording thermometer was kept in the compartment, as a rule. The temperature range was noted whenever cultures were removed from the freezer and the indices were reset. In some experiments, laboratory thermometers were kept in the compartment and the temperature was noted at frequent intervals. These thermometers and the metal back of the maximum and minimum thermometer were in contact with the metal coil in which the refrigerant circulated or with a thin layer of ice covering the coil. The bottoms and sometimes the sides of the bottles containing the feces were in contact with the same surface. The actual temperature of the feces was judged to be similar to that registered by the thermometers. It was noted, however, that the temperature of the air in the compartment was somewhat above that recorded by thermometers placed as has been described. A thermometer connected with the thermostat was frequently read to determine the variation in the temperature of the cooler.

A maximum and minimum thermometer was kept at the site of each outdoor experiment. The temperature range was noted each time that cultures were brought into the laboratory and the indices were reset.

The bottles involved in outdoor experiments, as well as the thermometer used to record temperatures, were in the shade at all times. Thus the feces were protected from direct sunlight. Since the bottles were covered, the feces were also protected from wetting by snow or rain and from appreciable desiccation. The possible inimical influences, therefore, so far as survival and development of the eggs and larvae are concerned, were largely limited to low temperatures and natural fluctuations in temperature.

The Baermann isolation apparatus was used throughout this investigation for the recovery of preinfective and infective larvae from feces. As noted elsewhere, in most experiments involving recovery of preinfective larvae, a piece of silk bolting cloth was placed on the screen to reduce the passive descent of fecal particles and dead larvae. Fluid was not withdrawn from the hose for at least 48 hours after the feces had been placed on the screen. Usually the period that elapsed was much longer. Descent of larvae into the stem of the funnel of the Baermann apparatus was the criterion of their viability. Most of the preinfective larvae were dead when the fluid was examined as these larvae usually live only a short time in water, even under favorable conditions. Successive withdrawals of fluid from the rubber hose attached to the stem of the funnel were continued until samples were found to be negative for larvae when inspected under the binocular microscope. Following a process of sedimentation and centrifugation, the larvae recovered were concentrated in a small volume of water and counted. The counts were direct or by means of a dilution method, depending on the abundance of the larvae. A dilution technique was used in making egg counts.

In certain experiments it was necessary to determine the developmental stages of the larvae recovered from cultures or to count preinfective and infective larvae differentially. As already indicated, most preinfective larvae died after remaining in contact with the fluid in the Baermann apparatus for 48 hours. Third-stage larvae, on the other hand, survived very much longer. Therefore, in general all larvae that were dead or partly disintegrated at the time of examination were counted as preinfective and all that were alive and of normal appearance were counted as third stage. However, the size, general appearance, and structure of the larvae were also used in differentiating them. These criteria were likewise used in distinguishing the various stages of development among preinfective larvae—the first stage, second stage, or second ecdysis. The appearance and organization of the intestine were used to differentiate partly developed from fully developed third-stage larvae, but if there was any doubt as to whether a larva should be considered infective or preinfective, it was classified as infective.

In the examination of some cultures, after the usual differential count with the dissecting microscope had been completed, 0.075 cc. of a suspension of the larvae was also examined under a compound microscope and a second differential count of the larvae was made. Sometimes the larvae were treated with an iodine solution to aid in determining their morphological status. Usually the total number of larvae recovered from the culture was then calculated from the results of the first count under the dissecting microscope and the numbers or percentages of larvae in the various developmental stages were calculated from the differential counts obtained by using the compound microscope. The detailed results of these examinations are too voluminous for inclusion with the experimental data. Unless otherwise indicated in the text or tables, all figures given in connection with experiments on preinfective larvae are based on differential counts obtained with the dissecting microscope only.

LABORATORY EXPERIMENTS WITH EGGS

EFFECT ON SURVIVAL OF UNEMBRYONATED EGGS OF CONTINUOUS EXPOSURE TO SUBFREEZING TEMPERATURES AND TO A MEAN TEMPERATURE OF 36° F.

As shown in table 1, in experiment 1 a small percentage of unembryonated eggs in a culture continuously frozen for 49 days were still alive, but in experiment 2 all eggs in cultures frozen for 48 days or longer were dead. A small percentage of eggs in feces frozen for 82 days were still alive in experiment 3.

TABLE 1.—*Survival of unembryonated horse strongyle eggs in feces continuously exposed, for indicated periods, to subfreezing temperatures and to a mean temperature of about 36° F.*

Experiment No.	Culture No.	Weight of feces	Period of exposure	Approximate range of temperatures at which cultures were exposed	Estimated mean temperature of entire exposure period	Infective larvae recovered	Apparent survival [1]
		Grams	*Days*	*°F.*	*°F.*	*Number*	*Percent*
1	1	75	7	14-30	21	17,700	92.9
	2		11	14-30		3,070	16.1
	3		21	14-30		7,150	37.5
	4		42	14-30		5,750	30.2
	5		[2] 49	14-30		1,110	5.8
	6		7	34-37	36	3,660	19.2
	7		14	34-37		12,520	65.7
	8		21	34-37		9,850	51.7
	9		62	34-37		8,000	42.0
	10		140	34-37		1,050	5.5
	11		(3)			28,300	--
	12		(3)			9,900	--
2	1	35	48	14-23	17	0	0
	2		54	14-23		0	0
	3		61	14-23		0	0
	4		68	14-23		0	0
	5		(3)			61,000	--
	6		(3)			52,200	--
	7		(3)			67,000	--
	8		(3)			51,600	--
3	1	35	14	10-30	14	2,500	8.7
	2		21	10-30		2,050	7.2
	3		31	8-30		440	1.5
	4		40	7-30		860	3.0
	5		56	6-30		735	2.6
	6		68	6-30		250	.9
	7		82	6-30		500	1.7
	8		24	34-37	36	1,675	5.8
	9		45	[4] 34-41		750	2.6
	10		68	[4] 34-44		3,850	13.4
	11		96	[5] 34-50		1,000	3.5
	12		114	[5] 34-50		1,200	4.2
	13		153	[6] 34-50		225	.8
	14		195	[6] 34-50		135	.5
	15		(3)			25,200	--
	16		(3)			31,200	--
	17		(3)			29,500	--
4	1	30	29	34-37	36	4,400	16.7
	2		84	[7] 34-46		400	4.2
	3		99	[7] 34-46		250	2.7
	4		126	[7] 34-46		185	2.0
	5		155	[7] 34-46		38	.4
	6		169	[7] 34-46		31	.4
	7		(3)			17,000	--
	8		(3)			1,280	--
	9		(3)			13,250	--
	10		(3)			9,100	--
	11		(3)			2,000	--
	12		(3)			13,600	--

[1] Based on the average number of infective larvae recovered from the control cultures.

[2] Actually 50 days, but the culture had thawed beginning about 24 hours earlier owing to an interruption in electrical service.

[3] Control; unexposed.

[4] Maximum attained in the course of an 18-hour period beginning on the 28th day of exposure.

[5] Maximum attained in the course of a 5-day period beginning on the 69th day, but 43° F. was exceeded during only a brief part of this period; also subjected to maximum as shown in footnote 4 for cultures 9 and 10.

[6] [illegible] corded in the course of intervals of not more than 18 hours; also subjected to maxima as shown in footnote 5 for cultures 11 and 12.

[7] Maximum attained in the course of an 18-hour period beginning on the 69th day of exposure.

Cultures containing unembryonated eggs were also subjected to continuous freezing in connection with the experiments reported in tables 2 and 4. A small percentage of eggs in a culture frozen for 97 days yeilded infective larvae, as shown in table 2. In the experiment reported in table 4, about 28 days was the maximum exposure and, as in all other experiments, an exposure of this duration was not lethal to all eggs.

The proportion of eggs surviving roughly comparable periods of exposure in these five experiments varied widely, as is indicated by the relationships between the number of infective larvae recovered from exposed and control cultures. One factor contributing to this result probably was variation in the total number of eggs in the feces comprising the cultures of each experiment. This is regarded as a principal cause of the wide variation in the number of infective larvae recovered from the various control cultures of some of the experiments. Difference in extreme and mean temperatures of exposure probably was an additional factor in determining the proportion of eggs surviving in comparably exposed test cultures.

The experiments were, therefore, of limited quantitative value. They definitely showed, however, that continuous exposure to subfreezing temperatures ultimately exerted a pronounced lethal effect on unembryonated eggs. That only a small proportion of eggs, or none at all, survived exposures of about 7 to 8 weeks at a mean temperature of about 14° to 21° F., is also a warranted conclusion. That at a given range of subfreezing temperature the number of surviving eggs decreased gradually as the duration of exposure increased, is strongly suggested by the data of these experiments as a whole.

In the experiments involving exposure of unembryonated eggs in feces to a mean temperature of about 36° F., very large proportions were killed by prolonged exposure, as shown in table 1. A small proportion survived an exposure of 195 days in one experiment. In the remaining two experiments the maximum periods of exposure were 140 and 169 days, and these exposures also did not kill all the eggs. In two of the three experiments, about one-half of the eggs were still alive after being kept at about 36° for 3 to 4 weeks. In general, the data strongly suggest that decrease in the number of viable eggs and increase in duration of exposure were related. But because of variability in results, the experiments do not warrant quantitative conclusions with respect to the rate of devitalization of the eggs.

EFFECT OF EMBRYONATION ON RESISTANCE OF EGGS TO SUBFREEZING TEMPERATURES

Embryonated eggs were much more readily killed than unembryonated eggs in feces exposed to the same range of subfreezing temperatures (7° to 32° F.), as shown in table 2. That cultures 7 to 12, after incubation in the laboratory for 19 hours, contained mainly embryonated eggs when exposed was shown by examination of an identically incubated culture of the same lot. Four grams of feces was removed from this culture for an egg count. From the remaining 26 gm. only about 500 preinfective larvae were recovered. The egg count showed that the feces contained about 900 eggs per gram, that a majority of the eggs contained larvae, and that many of the remaining eggs were in the tadpole stage of development. Since there was a small residue of segmenting eggs in the feces, these unembryonated eggs probably were the source of many of the infective larvae recovered from cultures 7 to 12 after the latter had been exposed and incubated at room temperature. The experiment does not permit a conclusion as to the absolute resistance of the embryonated eggs.

TABLE 2. *Comparison of the survival of unembryonated and embryonated eggs in 30-gm fecal cultures continuously exposed, for indicated intervals, to subfreezing temperatures*

CULTURES CONTAINING ONLY UNEMBRYONATED EGGS (16- TO 64 CELL STAGES)

Culture No.	Period of exposure	Approximate range of temperatures at which cultures were exposed[1]	Infective larvae recovered	Apparent survival[2]
	Days	*°F*	*Number*	*Percent*
1	13	9-26	2,950	31.3
2	29	7-26	725	7.7
3	34	7-26	2,370	25.2
4	47	7-32	850	9.0
5	56	7-32	230	2.4
6	97	7-32	34	4

CULTURES CONTAINING MAINLY EMBRYONATED EGGS[3]

Culture No.	Period of exposure	Approximate range of temperatures at which cultures were exposed[1]	Infective larvae recovered	Apparent survival[2]
7	11	9-26	1,000	10.6
8	28	7-26	170	1.8
9	33	7-26	50	.5
10	46	7-32	80	.8
11	55	7-32	38	.4
12	96	7-32	1	01

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[1] Estimated mean temperature of entire period of exposure, 16° F.

[2] Based on the average number of infective larvae recovered from the control cultures. The control cultures for this experiment were the same as for experiment 4 in table 1.

[3] See text.

EFFECT OF ALTERNATE FREEZING AND THAWING OF FECES

SURVIVAL OF UNEMBRYONATED EGGS AT SUBFREEZING TEMPERATURES

To determine whether a repetition of the physical changes involved in freezing and thawing of feces would affect the resistance of unembryonated eggs to subfreezing temperatures, the various test cultures were frozen, removed from the freezer, thawed at room temperature, and frozen again from 3 to 10 times before they were finally incubated in the laboratory. To prevent appreciable development of the eggs during the periods of thawings, the cultures were allowed to remain at room temperature only until all ice crystals had melted and the feces had attained their normal consistency.

Resistance of the eggs to a mean temperature of about 14° F. was not reduced by repeated freezing and thawing of the feces. This fact is indicated by comparison of the number of infective larvae recovered from test cultures (table 3) and from those prepared from the same lot of feces but continuously frozen at similar temperatures (table 2). The mean subfreezing temperature of exposure of the series that was alternately frozen and thawed was lower than that of the continuously frozen series.

It was impracticable to determine the actual temperature of the feces during the various thawings. However, it is evident from the results of the experiment that the procedure employed prevented appreciable development of the eggs during these periods, for embryonation decreases the resistance of the eggs to subfreezing temperatures, as has been shown previously.

TABLE 3. - *Survival of unembryonated eggs at subfreezing temperatures as affected by repeated freezing and thawing of 30-gm. cultures of feces*

Culture No	Total duration of exposure to subfreezing temperatures	Approximate range of temperatures at which cultures were exposed[1]	Times cultures were—		Intervals between (1) initial exposure to freezing and (2) thawings	Total time cultures were at room temperature for thawing	Period cultures were frozen after last thawing	Infective larvae recovered after incubation following final freezing	Apparent survival[2]
			Thawed	Frozen					
	Days	°*F*	*Number*	*Number*	*Days*	*Hours*	*Days*	*Number*	*Percent*
1	12.75	14-21	3	4	0.75, 6, 11.5	6.0	1.25	4,300	45.6
2	28.50	14-21	4	5	0.75, 6, 11.5, 14.75	7.5	13.75	3,000	31.8
3	33.50	10-21	5	6	0.75, 6, 11.5, 14.75, 29	9.0	4.50	1,600	17.0
4	46.50	10-21	6	7	0.75, 6, 11.5, 14.75, 29, 34	10.5	12.50	650	6.9
5	55.50	7-21	7	8	0.75, 6, 11.5, 14.75, 29, 34, 47	12.0	8.50	875	9.3
6	96.25	7-21	10	11	0.75, 6, 11.5, 14.75, 29, 34, 47, 56, 61.5, 83.75.	17.5	12.50	100	1.1

[1] Estimated mean temperature of entire exposure period, 14° F.

[2] Based on the average number of infective larvae recovered from the control cultures, the control cultures for this experiment were the same as for experiment 4 in table 1.

POTENTIAL YIELD OF INFECTIVE LARVAE

As previously reported, embryonated eggs were found to be more susceptible to subfreezing temperatures than unembryonated eggs. In experiments described later, nearly all preinfective larvae were killed by subfreezing temperatures in about 4 days and many were killed by much briefer exposures. These facts suggest that horse feces passed outdoors in regions where freezing and thawing alternate frequently in winter and early spring are largely "sterilized" during these seasons. It would seem that development would occur during periods of moderate temperatures and that succeeding subfreezing temperatures would readily kill the more susceptible stages mentioned. However, the degree of development and destruction would be determined by the relationships prevailing between the variables of temperature and time.

To obtain more information on this point, an experiment was performed in which feces originally containing unembryonated eggs were alternately frozen and thawed under laboratory conditions. This experiment differed from the freezing and thawing experiment already described in that the feces not only were thawed at room temperature but also were kept in the laboratory long enough to permit some development of the free-living stages each time that the cultures were removed from the freezer. The objective was to obtain the greatest possible lethal effect. Although outdoor fluctuations in temperature were simulated in the experiment, the transitions from subfreezing temperature to temperatures favorable for development were more rapid than those that occur in nature.

The yield of infective larvae from cultures subjected to repeated alternate freezing and thawing under the conditions of this experiment was reduced to a very small percentage of their potential yield, as shown in table 4, series C. In fact, culture 1 of series C, which was frozen for about 5 days, transferred to room temperature for 2 days, and again frozen for 5 days, yielded, on incubation, a negligible number

TABLE 4.—*Comparison of the effects of alternate freezing and thawing and of continuous freezing on the yield of infective larvae from 30-gm. cultures initially containing unembryonated eggs*

Culture No.	Times culture was frozen	Duration of freezing period No.—					Total period of freezing	Range of freezing temperature	Times culture was thawed	Duration of thawing period No.—				Total period of thawing	Larvae recovered		Yield of infective larvae as compared with potential yield [1]
		1	2	3	4	5				1	2	3	4		Pre-infective	Infective	
	Number	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	°*F.*	*Number*	*Days*	*Days*	*Days*	*Days*	*Days*	*Number*	*Number*	*Percent*
SERIES A.—ALTERNATELY FROZEN AND THAWED AND EXAMINED IMMEDIATELY FOLLOWING FINAL PERIOD OF THAWING																	
1	1	4.8					4.8	15–32	1	2.0				2.0	1,000	0	
2	2	4.8	4.9				9.7	15–32	2	2.0	1.1			3.1	81	57	
3	3	4.8	4.9	1.1			10.8	14–32	3	2.0	1.1	1.1		4.2	39	17	
4	4	4.8	4.9	1.1	1.8		12.6	7–32	4	2.0	1.1	1.1	3.0	7.2	4	19	
SERIES B.—ALTERNATELY FROZEN AND THAWED AND EXAMINED IMMEDIATELY FOLLOWING FINAL PERIOD OF FREEZING																	
1	2	4.8	4.9				9.7	15–32	1	2.0				2.0	7	0	
2	3	4.8	4.9	1.1			10.8	14–32	2	2.0	1.1			3.1	5	2	
3	4	4.8	4.9	1.1	1.8		12.6	10–32	3	2.0	1.1	1.1		4.2	4	1	
4	5	4.8	4.9	1.1	1.8	8.0	20.6	7–32	4	2.0	1.1	1.1	3.0	7.2	1	11	
SERIES C.—ALTERNATELY FROZEN AND THAWED AND EXAMINED FOLLOWING INCUBATION AFTER FINAL PERIOD OF FREEZING																	
1	2	4.8	4.9				9.7	15–32	1	2.0				2.0	0	92	1.0
2	3	4.8	4.9	1.1			10.8	14–32	2	2.0	1.1			3.1	0	50	.5
3	4	4.8	4.9	1.1	1.8		12.6	10–32	3	2.0	1.1	1.1		4.2	0	51	.5
4	5	4.8	4.9	1.1	1.8	8.0	20.6	7–32	4	2.0	1.1	1.1	3.0	7.2	0	33	.4
SERIES D.—FROZEN CONTINUOUSLY AND EXAMINED FOLLOWING INCUBATION AT ROOM TEMPERATURE																	
1	1	11.7					11.7	13–32	0					0	0	3,500	37.6
2	1	14.0					14.0	8–32	0					0	0	1,900	20.4
3	1	16.8					16.8	8–32	0					0	0	1,800	19.3
4	1	27.7					27.7	7–32	0					0	0	535	5.7
SERIES E.—UNEXPOSED CULTURES																	
1																8,500	
2																11,350	
3																10,500	
4																5,850	
5																10,600	
6																9,100	

[1] Based on the average number of infective larvae recovered from the control cultures.

of infective larvae. Evidently this culture contained principally embryonated eggs when frozen the second time, for culture 1 of series A, after also being frozen once and incubated in the laboratory for 2 days, yielded but a small number of preinfective larvae as compared with the average number of infective larvae recovered from the unexposed control cultures. Moreover, these preinfective larvae were nearly all killed by the second period of freezing, as shown by the results obtained for culture 1 of series B. A few infective larvae developed in the actual course of the alternate freezing and thawing treatment, as shown by the results obtained with cultures 2, 3, and 4, series A. Some of these infective larvae survived the various periods of freezing.

The recovery of comparatively large numbers of larvae from the continuously frozen cultures (series D) shows that the development, during the thawing periods, of stages more susceptible than the unembryonated egg was responsible for the far greater lethal effect observed in the cultures alternately frozen and thawed.

The incidental evidence of this experiment on the susceptibility to freezing of preinfective and newly developed infective larvae is in agreement with the results of later experiments. That this experiment also provides evidence on the resistance to freezing of unembryonated eggs has been previously pointed out.

OUTDOOR EXPERIMENTS WITH EGGS

Two experiments in which freshly passed feces were subjected to outdoor temperatures were carried on in the winter of 1938 39. The results of the first of these experiments are given in table 5. The egg counts on four of the control cultures of this experiment appeared to show a fairly even distribution of the eggs in the feces from which cultures were prepared. However, the number of infective larvae recovered from four other control cultures varied widely. Also, in the test cultures, there was no apparent relationship between the potential yield of infective larvae and the duration of exposure. Hence, the experiment failed to demonstrate quantitatively that the potential ability of the cultures to yield infective larvae was reduced by exposure to outdoor winter and spring temperatures.

Nevertheless, the following inferences from other data of the experiment are considered warranted: A few eggs in the feces became embryonated in the first 12 days of exposure, during which the maximum temperature recorded was 46° F., and many became embryonated and hatched in the first 22 days of exposure, during which 56° was the maximum temperature recorded. Embryonation was directly observed. That eggs hatched was indicated by a decrease of more than one-third in their number. Since a corresponding number of preinfective larvae was not found in culture 8, examined after 22 days of exposure, many larvae evidently were killed soon after hatching. Later in the course of the first 55 days of exposure, more eggs apparently hatched, but only a small number of larvae succeeded in reaching the infective stage. Hence, many more preinfective larvae apparently were killed during the last 33 days of this period. Thereafter, when temperatures more favorable for development were attained periodically, the number of viable eggs in the feces diminished further, and despite frequent alternations between subfreezing and moderate temperatures and rather extensive and rapid fluctuations in temperature, preinfective and infective larvae increased in number. The developmental process had not been completed when the experiment was terminated on April 7, after the feces had been exposed 113 days. There was no evidence that unembryonated or embryonated eggs were killed in appreciable numbers under the conditions of this experiment.

TABLE 5.—*Survival and development of free-living stages of horse strongyles in 35-gm. cultures of feces containing unembryonated eggs when placed outdoors on December 15, 1938*

Culture No.	Date culture was removed to laboratory	Duration of outdoor exposure	Range of temperature in each interval of exposure	Eggs in feces when removed to laboratory		Larvae recovered from culture when removed to laboratory			Infective larvae recovered after incubation of culture at room temperature for week or more
				Per gram	Condition	Preinfective	Infective	Total	
		Days	*°F*	*Number*[1]		*Number*	*Number*	*Number*	*Number*
1	*1938* Dec 27	12	25–46	1 150	A few embryonated, remainder apparently viable				
2									
3						[2] 115	0	[2] 115	1,900
4	*1939* Jan 2	17 7	14 56	1,000	About one-third embryonated remainder apparently viable				
5									
6						[2] 40	0	[2] 40	10,750
7	Jan 6	22	33–55	943	Majority embryonated, remainder apparently viable				
8									
9						38	0	38	
10	Jan 19	35	9 60	915	do				3,750
11						215	0	215	[4] 10,000
12									10 500
13	Feb 8	54 7	18–46	465	Majority embryonated, viability of remainder questionable	[4] 162	[4] 15	[4] 177	
14									7,350
15	Feb 28	75	20–70	50	Apparently nonviable	[4] 1,150	[4] 162	[4] 1,312	
16									1,925
17	Apr 7	113	22 78	50	do	[4] 26	[4] 544	[4] 570	
18									1,700
19	(5)								2,900
20	(5)								1,300
21	(5)								22 300
22	(5)								3 000

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[1] Egg counts on 4 control cultures were 1,370, 1,580, 1,580, 1,700 All figures are the average of counts on two 4-gm samples from each culture

[2] Regarded as hatching from embryonated eggs while the feces were in the Baermann apparatus.

[3] Approximate, being temperatures recorded 1 mile away (thermometer used in experiment accidentally jarred)

[4] 8 gm of feces had been removed from this culture for egg counts The actual number of larvae recovered from the remainder of the culture was adjusted to the 35-gm basis.

[5] Unexposed; control.

The most important facts shown by this experiment are as follows: Feces placed outdoors when fresh yielded appreciable numbers of infective larvae in the course of an exposure of about 3½ months to the fluctuating subfreezing and moderate temperatures prevailing in winter and early spring in the vicinity of Beltsville, Md., and were still a potential source of additional infective larvae early in April. From January 13 to 21, the cultures and the thermometer at the site of the experiment were partly or completely covered with snow. During this time the actual temperatures of exposure undoubtedly did not correspond with air temperatures. At no other time during this experiment were more than traces of snow on the ground.

It was observed at the site of the outdoor experiments that air temperatures below and above the freezing point often occurred during the same day and that sometimes transitions from moderate subfreezing temperatures to those fairly favorable for development took place within 6 or 7 hours. The exact frequency of such temperature fluctuations was not recorded, however. Daily maximum and minimum temperatures recorded by an instrument located about 1 mile from the site of the experiments indicated that subfreezing temperatures occurred on 83 of the 132 days from December 15, 1938, to April 26, 1939, the period that the outdoor experiments were in progress. On 77 of these 83 days, thawing temperatures also were recorded. Fluctuations from about 20° or 25° F. to about 50° or 60° during December and January and from a little below freezing to about 60° or 70° in February and March occurred several times within periods of about 8 to 24 hours. Since the maximum and minimum temperatures recorded on the thermograph tended to differ somewhat from those observed during the same periods at the site of the experiments, the facts mentioned are not admissible as data of the experiments. However, they indicate that the experimental results were obtained in an area where frequent alternations of freezing and thawing temperatures and violent and rapid fluctuations in temperature were characteristic of the period of the experiments.

In the second experiment, begun 42 days later than the first, the outdoor exposure caused a marked reduction by spring in the number of infective larvae that developed in cultures (table 6) as compared with the potential yield (controls in table 4). Cultures exposed 90 days ending April 26, 1939, yielded infective larvae representing, on the average, slightly less than 10 percent of the average number recovered from the six control cultures. Many of the results obtained in the first experiment also are applicable to this experiment. An appreciable decrease in the number of eggs in the feces took place in the initial 13 days of exposure, during which the maximum temperature was only 46° F. That nearly all larvae hatching in this period were promptly killed is indicated not only by the failure to find more than a few survivors, but also by the fact that the percentage of reduction in the potential yield of infective larvae (37 percent) was only a little less than the observed decrease in the number of eggs (42 percent).

In the ensuing period of 20 days (from February 8 to 28), characterized by alternations between temperatures a few degrees below freezing and those favorable for moderately rapid development, the potential yield of infective larvae was still more markedly reduced. As in the previous experiment, however, some infective larvae developed in this period. This was apparently due partly to the fact that temperatures remained continuously above the freezing point for considerable periods and partly to the fact that although freezing at intervals killed some preinfective larvae, the temperatures were not low enough or sufficiently prolonged to kill all of them.

TABLE 6.—*Survival and development of free-living stages of horse strongyles in 30-gm. cultures of feces containing unembryonated eggs when placed outdoors on January 26, 1939* [1]

Culture No	Date culture was removed to laboratory	Duration of outdoor exposure	Range of temperature in each interval of exposure	Eggs in feces when removed to laboratory		Larvae recovered from culture when removed from laboratory [2]			Infective larvae recovered after incubation of culture in laboratory for week or more
				Per gram	Condition	Preinfective	Infective	Total	
	1939	Days	°F	Number [3]		Number	Number	Number	Number
1	Feb 8	12.7	22-46	483	Some embryonated	12	0	12	
2									5,850
3	Feb 28	32.7	20-70	100	Apparently dead	370	7	377	
4									685
5	Mar 17	49.7	23-70	100	do	68	16	84	
6									850
7	Mar 24	57.0	22-78	100	do	200	4	204	
8									1,065
9	Apr 7	71.0	29-78	100	do	10	295	305	
10									960
11	Apr 26	90.0	[4] 30-88	-		0	1,050	1,050	
12						0	800	800	
13									690

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[1] The control cultures for this experiment were the same as for the experiment reported in table 4.

[2] Except for cultures 11 and 12 the data were calculated from the number of larvae recovered from 22 gm of feces after 8 gm had been removed from the culture for egg counts.

[3] Egg counts on 4 control cultures were 800, 900, 1,400, 1,600. All figures are the average of counts on two 4 gm samples from each culture.

[4] Approximate, being temperatures recorded 1 mile away (thermometer used in experiment accidentally jarred).

The experiment provides no definite evidence of pronounced lethal action later in the exposure period, but the number of infective larvae in the cultures did not reach a maximum until the onset of fairly continuous moderate temperatures. The data indicate that some eggs were killed in this experiment. Although all the eggs in cultures 3, 5, 7, and 9 were considered to be dead, the number of infective larvae recovered from the comparably exposed cultures 4, 6, 8, and 10, respectively, exceeded the number of preinfective and infective larvae in the former group.

The cultures of the previous experiment were exposed in part to the same conditions that clearly resulted in a reduction in yield of infective larvae in the cultures of the present experiment. That a similar result was not definitely apparent in the former experiment was probably due to uneven distribution of the eggs in the feces comprising the cultures.

LABORATORY EXPERIMENTS WITH PREINFECTIVE LARVAE

SURVIVAL OF LARVAE IN EARLY STAGES OF DEVELOPMENT

Three experiments were performed in which fecal cultures were kept at room temperature for 42 to 53 hours before being exposed to subfreezing temperatures. All larvae recovered from the control cultures placed in the Baermann apparatus when the test cultures were exposed were either first stage or second stage. In experiment 1, first-stage larvae predominated. In experiments 2 and 3 the two stages were about equally represented.

Considerably more than 90 percent of these preinfective larvae were killed in 1.2 to 4 days at a mean temperature of 14°-21° F., as shown in table 7. The results of experiment 1 are regarded as less accurate than those of experiments 2 and 3, since in examining the cultures of the latter two experiments a piece of silk bolting cloth was placed over the screen of the Baermann apparatus to minimize the descent of dead larvae. Larvae were recovered from cultures exposed much longer than 4 days, but this fact is attributed to the presence in the feces of a residue of viable eggs. Apparently some of these eggs hatched while the feces were in the Baermann apparatus. As shown in table 7, culture 1 of experiment 2 yielded only 150 surviving larvae, but when culture 2, which was exposed slightly longer, was incubated in the laboratory for a week it yielded more than 1,000 infective larvae. This finding indicates that the cultures of this experiment contained about 1,000 eggs that had not undergone appreciable development when the test cultures were exposed. It is well known that the development of the free-living stages of strongyles does not proceed at precisely the same rate in all parts of an ordinary culture, and some viable eggs still may be present in a culture that contains infective larvae.

TABLE 7.—*Survival of preinfective larvae, in early stages of development, in 70-gm. cultures of feces continuously exposed to subfreezing temperatures or at about 36° F.*

Experiment No.	Culture No.	Period of exposure	Approximate range of temperatures at which culture was exposed	Estimated mean temperature of entire exposure period	Larvae recovered [1]	Apparent survival [2]
		Days	*° F.*	*° F.*	*Number*	*Percent*
1	1	4.0	14–30	19	1,500	5.3
	2	10.1	14–30		[3] 2,775	9.7
	3	17.9	14–30		1,280	4.5
	4	19.0	14–30		[4] 260	----
	5	[5] 25.0	14–30		16	.06
	6	4.0	34–37	36	21,700	76.0
	7	17.9	34–37		26,400	92.5
	8	38.1	34–37		7,100	24.9
	9	80.1	34–37		1,900	6.7
	[6] 10	115.9	34–37		[7] 3	.01
	[6] 11	130.1	34–37		52	.2
	12	([8])	----	----	32,000	----
	13	([8])	----	----	25,100	
2	1	3.8	14–32	21	150	.4
	2	4.0	14–32		[4] 1,050	----
	3	6.0	14–32		10	.02
	4	11.9	14–32		26	.06
	5	19.0	14–32		15	.04
	6	33.8	14–32		7	.02
	7	47.8	14–32		2	.005
	8	58.1	14–32		0	0
	9	([8])	----	----	40,300	----
	10	([8])	----	----	[4] 47,500	----
3	1	1.2	7–18	14	8,775	4.4
	2	1.3	7–18		2,700	1.4
	3	4.1	7–18		29	.01
	4	5.0	7–18		67	.03
	5	13.1	7–18		122	.06
	6	13.1	7–18		55	.03
	7	([8])	----	----	194,000	----
	8	([8])	----	----	205,000	----

[1] Preinfective unless otherwise noted.

[2] Based on the average number of larvae recovered from the control cultures.

[3] Culture placed in Baermann apparatus 18 hours after removal from freezer.

[4] Infective larvae, culture incubated at room temperature for a week or more before examination.

[5] Actually 26 days, but electrical service was interrupted on the 25th day and culture had thawed when it was removed.

[6] Culture moistened on 80th day of exposure.

[7] Infective larvae developing during exposure.

[8] Unexposed; control.

Also in experiment 1, identically incubated cultures were exposed to a mean temperature of about 36° F. More than 90 percent of the larvae in a culture exposed for 80 days to this temperature died, as shown in table 7. Probably the cultures remaining in the cooler at this time were moistened too much, for few larvae were recovered from them when they were examined thereafter. A large proportion of the larvae survived for 18 days at this temperature, and approximately one-fourth survived for 38 days. Exhaustion of the food granules of the intestinal cells was noted in larvae surviving prolonged exposure. A few larvae reached the infective stage in a culture kept for 116 days at about 36°. Similar results were obtained in the first series of cultures of the experiment reported in table 9, although in that experiment somewhat larger percentages of larvae survived rather comparable exposures and more infective larvae developed while the cultures were kept at about 36°. However, only a general similarity in the results of the two experiments could be expected because the cultures of the first series in table 9 initially contained some infective larvae and some larvae in the second ecdysis.

SURVIVAL OF LARVAE IN ADVANCED STAGES OF DEVELOPMENT

Very large proportions of larvae principally in advanced phases of the second stage or in the second ecdysis, but not yet infective, were killed by comparatively brief exposure to subfreezing temperatures, as shown in table 8 Infective larvae in the cultures also were not very resistant to such temperatures. It was not anticipated that appreciable development of the larvae would occur at a mean temperature of about 36° F. Hence in examining the control cultures the larvae were separated into only two groups. Those that were obviously still preinfective and those regarded as infective were differentially counted; larvae regarded as of doubtful infectivity were included in the latter group. Similarly, in the classification and counting of the larvae from exposed cultures, larvae of doubtful infectivity were included with those definitely infective. In examining cultures 8, 9, and 10, exposed for about 16 to 33 days to a mean temperature of 36°, it was noted that the number of larvae that were infective or doubtfully infective had markedly increased over those in the control cultures. In the cultures kept at a mean of 36° for 3 months or longer, not only had a large percentage of larvae survived but nearly all definitely had reached the infective stage. The number of infective larvae recovered from culture 11 practically equaled the average number of larvae of all stages recovered from the control cultures. Temperatures above 37° were attained during only a few hours of the exposure period.

Another experiment was performed to test these results. The survival and development of larvae in a series of cultures containing mainly first-stage and second-stage larvae and one containing mainly larvae undergoing the second ecdysis were compared. Cultures of the first series contained a small number of infective larvae when exposed, but about 18 percent of the larvae in cultures of the second series were infective when exposed, as indicated by examination of a control culture of each series. The number and proportion of infective larvae increased in cultures of both series while the feces were kept at a mean temperature of about 36° F., as shown in table 9. This result is in

TABLE 8.—*Survival of preinfective larvae, in advanced stages of development, in 35-gm. cultures of feces incubated at room temperature for 74 hours before exposure to subfreezing temperatures or at about 36° F.*

Culture No	Period of exposure	Approximate range of temperatures to which culture was exposed	Estimated mean temperature of entire exposure period	Larvae recovered			Apparent survival [2]
				Preinfective [1]	Infective	Total	
	Days	*°F*	*°F*	*Number*	*Number*	*Number*	*Percent*
1	1.74	14-20	16	830	[3] 170	1,000	15.5
2	2.66	14-20		150	50	200	3.1
3	3.74	14-20		400	65	465	7.2
4	15.73	14-20		0	4	4	.06
5	17.68	14-20		1	31	32	.5
6	17.66	14-20		0	26	26	.4
7	30.71	7-20		[4] 23	28	51	.8
8	15.74	34-37	36	1,630	[3] 5,720	7,350	113.7
9	22.66	34-37		115	[3] 1,010	1,125	17.4
10	32.92	34-37		350	[3] 3,150	3,500	54.1
11	88.75	[5] 34-42		225	6,225	6,500	100.5
12	88.75	[5] 34-42		500	3,790	4,290	66.3
13	142.87	[5] 34-42		0	2,680	2,680	41.4
14	(6)			5,550	[4] 650	6,200	
15	(6)			6,550	[3] 650	7,200	
16	(6)			5,500	[3] 500	6,000	

[1] Mainly in advanced phases of the second stage and in various phases of the second ecdysis, unless otherwise noted

[2] Based on the average number of larvae of all stages recovered from the control cultures

[3] Including some doubtfully infective larvae, but proportion undetermined

[4] Living first-stage larvae regarded as having hatched from viable eggs while the feces were in the Baermann apparatus

[5] During a period of about 12 hours on the 73d day of exposure, electrical service was interrupted and the temperature of the cooler rose for a few hours to the maximum shown

[6] Unexposed, control

TABLE 9—*Comparison of survival of preinfective larvae in two series of 30-gm cultures of feces incubated for different periods at room temperature before exposure to subfreezing temperatures and to about 36° F*

INCUBATED IN LABORATORY FOR 50 HOURS AND CONTAINING MAINLY FIRST-STAGE AND SECOND-STAGE LARVAE WHEN EXPOSED

Culture No	Period of exposure	Approximate range of temperatures to which culture was exposed	Estimated mean temperature of entire exposure period	Larvae recovered [1]			Apparent survival [2]	Relation between number of infective larvae recovered from test and control cultures
				Preinfective	Infective	Total		
	Days	*°F*	*°F*	*Number*	*Number*	*Number*	*Percent*	*Percent*
1	1.9	8-24	15	1,350	52	1,402	4.3	80.0
2	2.7	8-24		400	20	420	1.3	30.8
3	3.8	8-24		28	2	30	.1	3.1
4	14.9	34-37	36	30,900	100	31,000	95.1	153.8
5	43.7	[3] 33-50		16,135	815	16,950	52.0	1,253.8
6 [4]	93.7	[3] 33-50		980	120	1,100	3.4	184.6
7	(5)			32,535	65	32,600		

INCUBATED IN THE LABORATORY FOR 73 HOURS AND CONTAINING MAINLY LARVAE IN THE SECOND ECDYSIS WHEN EXPOSED

Culture No	Period of exposure	Approximate range of temperatures to which culture was exposed	Estimated mean temperature of entire exposure period	Preinfective	Infective	Total	Apparent survival	Relation between number of infective larvae recovered from test and control cultures
8	1.0	8-24	15	950	1,100	2,050	13.6	40.0
9	2.0	8-24		970	550	1,520	10.1	20.0
10	3.8	8-24		53	23	76	.5	.8
11	13.9	34-37	36	12,250	6,400	18,650	123.5	232.7
12	42.8	[3] 33-50		8,400	7,500	15,900	105.3	272.7
13 [4]	92.8	[3] 33-50		2,625	4,000	6,625	43.9	145.5
14	(5)			12,350	2,750	15,100		

[1] Total numbers of larvae recovered from cultures 5, 7, 12, 13, and 14 were calculated from the usual dilution counts by the use of the dissecting microscope, but the numbers of preinfective and infective larvae were calculated from differential counts obtained by the use of the compound microscope.

[2] Based on average number of larvae of all stages recovered from control culture

[3] Early in the third week of exposure a maximum of 43° F was reached during an 18-hour interval, and during a 5-day period in the 6th week of exposure a maximum of 50° was reached for a few hours, but during most of this period the temperature ranged from 34° to 37°.

[4] Moistened with water on the 67th day of exposure.

[5] Unexposed; control.

general agreement with those of previous experiments. Owing to unavoidable circumstances the maximum temperature was somewhat higher in the present experiment.

Observations not included in the table also indicated that about 1.4 percent of the larvae in cultures of the first series and about 50 percent of the larvae in cultures of the second series were in a late phase of the second ecdysis when exposed. During exposure to a mean temperature of about 36°, the percentage of larvae in this phase increased in cultures of the first series and decreased in those of the second. This finding is further evidence of development at that temperature. The proportions of larvae surviving comparable exposures at this temperature were considerably higher in the second series of cultures. Apparently this result was due to the greater ability of larvae in the second ecdysis to complete their development to the infective stage at a mean temperature of about 36°. The survival in cultures 6 and 13 was lower than was expected; apparently too much water had been added to these cultures on the sixty-seventh day of exposure. First-stage and second-stage larvae apparently were more susceptible to the effects of excess moisture than the more advanced stages. That nearly all preinfective larvae in all stages are killed by moderate subfreezing temperatures in less than 4 days was confirmed by this experiment. The incidental observation that larvae which have just attained the infective stage are readily killed by freezing was also confirmed.

OUTDOOR EXPERIMENTS WITH PREINFECTIVE LARVAE

In an experiment begun in February 1939, cultures originally containing large numbers of preinfective larvae were exposed for 14 to 71 days to outdoor temperatures. The total number of preinfective and infective larvae recovered from exposed cultures immediately on removal to the laboratory, or the number of infective larvae obtained from comparably exposed cultures incubated in the laboratory before examination, represented about 4.5 to 25 percent of the average yield of larvae from four unexposed control cultures, as shown in table 10. Two of the exposed cultures (Nos. 8 and 9) yielded almost as many larvae as one of the control cultures (No. 11). However, as a whole, the experiment indicated that the prevailing outdoor temperatures had a pronounced lethal effect on preinfective larvae. Culture 1, which was exposed 14 days, contained more surviving preinfective larvae than any of the test cultures examined after longer exposure. The preinfective larvae from this culture represented, however, only about 2.6 percent of the average number of larvae found in the control cultures. There was no definite correlation between increase in duration of exposure and increase in the number of infective larvae developing in exposed cultures. Therefore, the lethal action against preinfective larvae in this experiment undoubtedly occurred mainly during the first 14 days of exposure, from February 14 to 28. Apparently, the development of infective larvae also occurred mainly during this period. In one of the experiments with eggs, a pronounced lethal effect was also observed to occur during a 20-day period ending on February 28.

TABLE 10.—*Survival and development of free-living stages of horse strongyles in 50-gm. cultures of feces containing preinfective larvae when placed outdoors on Feb. 14, 1939*

Culture No.	Date culture was removed to laboratory	Duration of outdoor exposure	Range of temperature in each interval of exposure	Larvae recovered from culture when removed to laboratory			Infective larvae recovered after incubation of culture in laboratory for week or more	Apparent survival[1]
				Preinfective	Infective	Total		
	1939	*Days*	*°F*	*Number*	*Number*	*Number*	*Number*	*Percent*
1	Feb 28	14	20-70	1,000	1,450	2,450	5,650	6.4
2								14.9
3	Mar 17	30 7	23-70	500	6,000	6,500	4,600	17.1
4								12.1
5	Mar 24	38	22-78	210	2,260	2,470	1,700	6.5
6								4.5
7	Apr 7	52	29-78	0	4,500	4,500	8,200	11.8
8								21.6
9	Apr 26	71	[2] 30-88	0	9,400	9,400		24.7
10	(3)						[4] 39,600	
11	(3)						[4] 10,450	
12	(3)						[5] 58,400	
13	(3)						[5] 13,700	

[1] Based on the average number of larvae recovered from the control cultures

[2] Approximate, being temperatures recorded 1 mile away (thermometer used in experiment accidentally jarred).

[3] Unexposed, control

[4] Examined March 22

[5] Preinfective larvae, recovered when test cultures were exposed.

Some of the preinfective and infective larvae recovered from exposed cultures and some of the infective larvae which developed in exposed cultures incubated in the laboratory probably originated from eggs which had not yet hatched at the time that the feces were placed outdoors. Although this experiment provides evidence that, under the temperature conditions which prevailed, most of the preinfective larvae were killed before they reached infectivity, it is of equal importance that the fluctuating winter and spring temperatures occurring during a 71-day period did not "sterilize" feces originally containing mainly preinfective larvae of horse strongyles. At no time during this experiment was there an appreciable depth of snow on the ground.

DISCUSSION

The results of the writer's laboratory experiments on the survival of unembryonated eggs in feces continuously exposed to freezing are most nearly comparable to those reported by Enigk (*5*), since they showed that a large proportion of the eggs kept at mean temperatures of 14° to 21° F. (about −10° to −6° C.) were killed in 47 to 56 days. Since quantitative methods were used and the experiments were continued for longer periods than those reported by Enigk, the resistance of the eggs to continuous freezing has been better defined by the writer's work.

The reports of previous investigators do not afford a suitable basis for comparison with the results of the writer's laboratory experiments on the resistance of preinfective larvae to freezing. The absolute and comparative resistance of these larvae to moderately severe freezing has been determined by this investigation. De Blieck and Baudet's (*4*) observations afforded no comparison between the resistance of eggs and preinfective larvae. The experiments of

Wetzel (*13*) and Parnell (*10*) did not establish the absolute resistance of the preinfective larvae. Those of the latter worker permitted only a general comparison between their resistance and that of the other stages.

Experiments closely comparable to those reported in the present paper on the effect, on the eggs and preinfective larvae, of prolonged exposure to a mean temperature of about 36° F. (2° C.) also do not appear to have been reported by other investigators. As previously noted, Gackstatter (*6*) and De Blieck and Baudet (*4*) found that eggs were uninjured by brief exposure to similar temperatures. The writer's experiments appeared to show that even brief exposures killed some eggs.

The writer noted that first-stage and early second-stage larvae surviving prolonged exposure at about 36° F. had very pale intestinal cells. Probably larvae of these stages are not directly injured by temperatures a few degrees above the freezing point but die as a result of starvation. Late second-stage and noninfective third-stage larvae were able to continue their development to the infective stage at such temperatures. Thus, the survival of preinfective larvae was evidently conditioned by the degree of their dependence on bacterial food or, from a slightly different viewpoint, by their morphological status.

Parnell (*10*) and Britton (*3*) mentioned 10° C. (approximately 50° F.) as the approximate minimum temperature at which horse strongyle eggs and first-stage larvae can develop. The experimental basis for these statements was not specified by either writer. Wetzel (*13*) stated that the lowest temperature at which infective larvae of *Strongylus equinus* developed in about 350 hours was 12° to 13° C. (approximately 54° to 56° F.). About 10 percent of the hatched larvae became mature. However, in outdoor experiments of the writer's investigation, eggs became embryonated and apparently hatched in a period in which the maximum temperature did not exceed 46° F. (approximately 8° C.). Also, as previously mentioned, De Blieck and Baudet (*4*) noted that eggs kept for 12 days at 6° C. contained viable embryos. Hueber (*7*) reported that "sclerostome" eggs hatched in about 20 days at 5° to 10° C. The writer's observation that preinfective larvae reached infectivity during long exposure at a mean temperature of about 36° F. (maximum 50° F.) is not regarded as contradictory to Wetzel's report because greater time intervals were involved. Also, whether larvae of *S. equinus* were among those undergoing development was not determined.

The writer's incidental observation that third-stage larvae were not very resistant to subfreezing temperatures immediately after becoming infective presumably is in line with the statement of Zavadovskii and Vorob'eva (*14*) that the resistance capacity of *Strongylus equinus* larvae increases with age, 7-day-old larvae being more resistant to freezing than 4-day-old larvae. The English summary of these authors does not definitely state that the 4-day-old larvae were infective, however.

Parnell's (*9*) statement that embryonated horse strongyle eggs are much less resistant to freezing than unembryonated eggs has been confirmed. The data of this investigation do not permit comparison of the resistance of embryonated eggs and preinfective larvae.

In the light of the writer's experiments, Parnell's (*10*) statement that frequent alternate freezing and thawing can be relied on to kill the free-living stages of horse strongyles requires qualification as does the previously quoted statements on this subject by Britton (*3*). The possible combinations of the variables temperature and time, as determinants of the lethal or nonlethal effect of alternations in freezing and thawing weather, are so numerous and complex that it is doubtful whether they can be reduced to a simple yet sufficiently inclusive verbal statement. The conditions stipulated by both writers were fulfilled in the outdoor experiments of the present investigation, but the free-living stages, including preinfective larvae, were not all killed and considerable numbers of infective larvae developed and survived. That alternate freezing and thawing weather may markedly reduce the potential yield of infective larvae in feces subjected to it is shown by this investigation. That suitable alternations are capable of causing virtually complete "sterilization" of feces originally containing eggs is also affirmed. But it is amply shown that the mere occurrence of frequent alternation of thawing and freezing weather affords no guarantee of sterilization.

Parnell's (*10*) later statement as to the susceptibility of all free-living stages to "violent and frequent fluctuations in temperature" differs somewhat from that just discussed. In the writer's experiments, infective larvae developed in feces originally containing eggs or preinfective larvae despite exposure to temperatures ranging from minima of 13° and 20° F., respectively, to a maximum of 88°, and despite repeated and rapid transitions from subfreezing to moderate temperatures.

With the exception of those reported in this paper, the only laboratory experiments known to the writer on the effects of alternate freezing and thawing on free-living stages of horse strongyles are those reported by Ober-Blöbaum (*8*), which involved infective larvae.

There is apparent disagreement between the findings of the present investigation and those of Parnell in the following respect: The writer found that large proportions of unembryonated eggs invariably were killed by continuous exposure to subfreezing temperatures for 7 to 8 weeks and that many evidently succumbed to much briefer exposure in laboratory experiments. Parnell (*9*), however, found that the continual low temperatures of a severe Canadian winter were insufficient to kill "sclerostome" eggs. Parnell's (*9*) statement that eggs exposed all winter embryonated and hatched normally in the spring suggests that none were killed. However, in the writer's opinion, the data of the experiment reported later by him (*10*) do not support such an interpretation.

The previously mentioned report of Baker, Salisbury, and Britton (*2*) appears to be more in agreement with the results of laboratory experiments, at least insofar as it tends to show that winter temperatures exert a lethal effect on horse strongyle eggs. However, although these authors reported the percentages of eggs killed by 65, 90, and 150 days of exposure in winter at Ithaca, N. Y., they did not include dates or temperature data, and the method of examination of the exposed feces serving as a basis for their calculations was not clearly described. For these and other reasons, it is difficult to evaluate the significance of the percentage relationships reported by these authors.

The writer's outdoor experiments did not show that winter temperatures near Beltsville had an appreciable lethal effect on horse strongyle eggs as such. But the temperature conditions were very different from those of the laboratory experiments, which demonstrated that prolonged and continuous exposure to subfreezing temperatures kills the eggs. Results similar to those of these laboratory experiments would occur outdoors only in regions where subfreezing temperatures prevail continuously for long periods in winter and hence closely parallel the experimental temperature conditions.

The protection against extremes of temperature afforded to organisms on the soil surface by a covering of snow has generally been overlooked by those who have investigated the survival of the free-living stages of strongyles of horses and other domestic animals under winter conditions. Swales (*11*) found that, in the presence of an appreciable blanket of snow, temperatures recorded at the soil surface continuously approximated the freezing point and varied only slightly despite fluctuations in air temperature. He concluded that winter air temperatures cannot be considered as having any direct effect on organisms on the soil surface, so long as a covering of snow is present. The organisms are to be regarded as existing in a relatively constant climate with temperatures approximating the freezing point. The ground was covered with snow for a short time during only one of the three outdoor experiments reported in this paper.

In a discussion of the influence of frost on larvae in pastures, Taylor (*12*) stated that the cessation of the development of infective larvae during a cold period must result in the storage of potential infective material and, when warmer weather comes, a mass development of infective larvae probably occurs. Taylor referred to conditions under which grazing animals constantly deposit feces on a pasture during the cold period. However, the writer's experiments appear to indicate definitely that the proportion of eggs that ultimately yield infective larvae in horse feces exposed during cold seasons is determined by the particular relationships prevailing between the variables of temperature and time.

It is well known that the Baermann apparatus is of limited efficiency for the recovery of larvae from feces. However, it was used in this investigation because it is the most practicable available criterion of viability of such larvae. It was not determined whether the preinfective larvae recovered from feces following exposure were necessarily capable of completing their preparasitic development since it was impracticable to attempt to establish this point. Whether infective larvae developing in feces during or after exposure to low temperatures were capable of normal parasitic development also was undetermined. The writer is unaware of any method whereby this question might have been resolved satisfactorily.

More accurate mathematical results probably could have been obtained in laboratory experiments had counted numbers of eggs and preinfective larvae been exposed to low temperatures in water. But the significance of results obtained in this way would have been questionable. Feces are the natural medium of these stages for the most part, and the type of medium apparently affects experimental results. Thus, Zawadowsky and Vorobieva (*14*) found that infective larvae of *Strongylus equinus* showed greater resistance if frozen in damp sand or feces than if frozen in a dry condition or in water. The difficulty of determining whether larvae originating from eggs exposed in water could complete their development also would have been introduced had this alternative medium been employed. Thus, it was felt, that despite the probability of variability in results, the use of feces, the natural medium, was preferable for the purposes of this investigation.

SUMMARY AND CONCLUSIONS

To determine the effects of continuous low temperatures, alternate freezing and thawing, and natural fluctuations in temperature in winter and early spring on the survival and development of horse strongyle eggs and preinfective larvae, laboratory and outdoor experiments were performed at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., in 1938 and 1939.

From 94 to 100 percent of unembryonated horse strongyle eggs in feces continuously exposed to mean temperatures of about 14° to 21° F. were killed in 47 to 56 days in laboratory experiments. The percentages of eggs surviving comparable exposures varied, and the absolute limit of the ability of the eggs to survive at the experimental temperature ranges was not established. In one experiment, 0.4 percent of the eggs survived 97 days of continuous exposure to subfreezing temperatures. The experiments suggested that the number of surviving eggs decreased gradually as the duration of exposure to subfreezing temperatures increased.

Embryonated eggs were much more readily killed than unembryonated eggs in feces kept at the same range of freezing and subfreezing temperatures (7°—32° F.). The absolute resistance of embryonated eggs was not determined

The lethal effect on unembryonated eggs subjected to the following treatment was no greater than that obtained by continuous freezing: Repeated exposure to a mean temperature of 14° F., alternated with thawings at room temperature for periods only of sufficient duration to allow the feces to attain their normal consistency. When the thawings at room temperature were of sufficient duration to permit appreciable development, the cultures were promptly and almost completely sterilized so far as yielding infective larvae was concerned.

The limit of the ability of eggs to survive at a mean temperature of about 36° F. was not determined. In one experiment 0.5 percent survived an exposure of 195 days. In two of three experiments about one-half of the eggs survived for 3 to 4 weeks at this mean temperature, but because of variability in results, quantitative conclusions as to the rate of devitalization at this temperature were not warranted. Apparently, decrease in the number of surviving eggs and duration of exposure were related.

More than 90 percent of preinfective larvae principally in the first stage and early second stage of development were killed in 1.2 to 4 days in feces exposed to mean temperatures of 14°–21° F. Preinfective larvae in more advanced phases of development did not differ markedly from the younger larvae in ability to withstand subfreezing temperatures. Preinfective larvae in all phases of development were far more susceptible to subfreezing temperatures than the unembryonated eggs. Larvae that had just reached the infective stage were readily killed by subfreezing temperatures.

About 75 to 95 percent of preinfective larvae in cultures containing mainly first-stage and second-stage larvae survived at a mean temperature of about 36° F. for periods up to approximately 2 weeks. About 25 to 50 percent survived for approximately 5 to 6 weeks. More than 90 percent died in about 3 months at this temperature, and those surviving prolonged exposure had very pale intestinal cells. Death of the larvae was attributed to starvation rather than to a direct injurious action of the temperature. The proportion of infective larvae in cultures originally containing principally larvae in the second ecdysis and advanced phases of the second stage increased markedly on prolonged exposure of the cultures at a mean temperature of about 36°. In these experiments the larvae surviving about 3 months of exposure represented 40 to 100 percent of the number of larvae of all stages originally present in the cultures.

When feces originally containing unembryonated eggs were exposed outdoors to winter and early spring temperatures in the vicinity of Beltsville, Md., some infective larvae developed during the winter and the feces were still capable of yielding additional larvae in the spring. One experiment was inconclusive as to the lethal effect of outdoor exposure, but in another experiment the number of infective larvae ultimately developing in exposed cultures represented about one-tenth of the number recovered from unexposed control cultures. Infective larvae also developed in winter in feces that contained preinfective larvae when exposed. The number of infective larvae that ultimately developed in exposed cultures or that such cultures were capable of yielding after exposure represented one-fourth to one-twentieth of the potential yield of the cultures. Eggs embryonated and hatched outdoors in a period in which the maximum temperature recorded was 46° F. During the outdoor experiments in which these results were obtained, the temperatures frequently fluctuated above and below the freezing point. Such fluctuations in winter and spring temperatures reduced the yield of infective larvae in exposed cultures but did not "sterilize" the cultures. The degree of the lethal effect of outdoor exposure in winter and spring depends on the relationships that prevail between the variables of temperature and time.

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INHERITANCE OF REACTION TO COMMON SCAB IN THE POTATO[1]

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INTRODUCTION

In the process of developing improved scab-resistant varieties of potatoes (*Solanum tuberosum* L.), it was found that individuals with similar clonal reactions often differed significantly in their breeding value. This led to an attempt to place the parental material into genetic groups based on the classification for scab reaction of their sexual progeny and to isolate the more desirable scab-resistant genotypes. The presentation includes four related series:

1. A study of a cross for the purpose of supplying a genetic classification that could be applied to the parental material. In this cross segregates and their sexual progenies from the F_1, F_2, and F_3 generations of a cross were utilized.
2. A study of parental material, consisting of 118 varieties or selections which were classified on the basis of the scab reaction of the sexual progeny into the same probable genotypes as were employed for the segregates from the above cross.
3. A study of the scab reaction of the hybrid progenies obtained by crossing certain of the above parental genotypes.
4. A study of the association between the color gene *P* and the reaction to scab.

MATERIAL

In 1924 a cross was made between Accession 123 and Lookout Mountain. Accession 123 was an unidentified clone obtained as a rogue in a field of Irish Cobblers. Both parents were self-fruitful, and the resulting hybrids were highly so. Under the plan of saving the seed in the breeding plots of all self-fruitful individuals, seed of a relatively large number of individuals from the F_1, F_2, and F_3 generations accumulated, though most of the individuals which produced this seed were discarded. When the studies on resistance to common scab were initiated, it was found that selections from this cross exhibited a wide range of scab reaction. Hence, this cross appeared to provide excellent material for a genetic classification of scab reaction. Recourse was then made to the seed which had accumulated from the hybrids of this cross during the previous 12 years. The segregates have each a pedigree number which shows their relationship and, by the number

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of numerals, the generation from which they were selected. Thus 5 is the cross number and is common to all the segregates. Similarly, 5–14 is an F_1 segregate whose progeny carries the same number, while 5–14–1, 5–14–2, 5–14–3 . . . are F_2 segregates from this progeny.

Parental material studied represented a cross-section of the self-fruitful clones in the breeding plots. It consisted of American and European varieties, and of selections from crosses and selfed lines. The pedigree numbers for the selections supply the same information as those previously indicated for the cross. The character of russeting was almost entirely absent in this parental material. Hence, the influence of russeting on reaction to scab as found in such varieties as Russet Burbank and Russet Rural was not considered.

METHODS

Relative resistance to common scab was determined by growing progenies in peat soil which was heavily infested with *Actinomyces scabies* (Thax.) Güss. as shown by the high degree of infection on certain individuals. Field tests were made in the first clonal generation except for one test in which certain progenies were retested in their second clonal generation. Another test was made by growing seedlings directly from seed on nonsterilized soil from the above field to which a single race of *A. scabies* was added. These progenies were tested in the field during the following summer.

Resistance was determined by the "highest scab" method as described by Leach et al.[2] According to this method, depth and severity of the scab lesion were used as a measure of susceptibility. Four degrees of severity were recognized, and these were indicated by numbers from 1 to 4 inclusive. The tubers of each individual of a given progeny were examined and the individual given a rating corresponding to the most severe type of pustule found. These ratings were then averaged to obtain the mean scab ratings. Each family was grown in two randomized blocks, and the interaction of families with blocks was used to obtain an estimate of the experimental error of the means.

It should be pointed out that the progenies used in this investigation were studied under limited conditions with respect to scab infection. Schaal[3] and Leach et al.[4] have shown that there are races of *Actinomyces scabies* which differ a great deal in pathogenicity on some lines of potatoes and that the same variety or seedling may differ in resistance when tested in different localities. Schaal[5] found that in general highly resistant varieties are consistently resistant when grown in widely different parts of the country, but that less resistant varieties may vary considerably. It was also pointed out by Wingerberg[6] that physiologic as well as morphologic factors may determine resistance to scab. Leach et al.[4] came to the same conclusion. This suggests the possibility that under a different set of conditions, especially with respect to the strains of the pathogen present, breeding behavior for resistance other than that reported in this paper may be found.

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In the analysis of the data, cognizance was taken of the evidence supplied by Lunden [7] indicating that the potato was an autotetraploid. Hence, for a character conditioned by a single gene there would be five possible genotypes.

With this type of inheritance, the material did not appear to be sufficiently extensive to warrant testing more than a simple hypothesis. Therefore the analysis was made on the assumption that among the possible genes influencing scab reaction there might be one with a major influence which would allow a genetic classification of the material. The classification into the five possible genotypes designated as Sc_4, Sc_3sc, Sc_2sc_2, $Scsc_3$, and sc_4 was made by inspection of the distribution of the progeny means for regions of discontinuity.

EXPERIMENTAL RESULTS

REACTION OF PROGENIES OF ACCESSION 123 × LOOKOUT MOUNTAIN

The sexual progeny of 14 F_1, 32 F_2, and 18 F_3 segregates of the cross Accession 123 by Lookout Mountain were tested for reaction to common scab. Their progeny means were then compared irrespective of generation for indications of the possible presence of five breeding types. The discontinuity in the distribution of the progeny means appeared to be greatest in four regions. A separation of the progeny means was made at these four regions, the segregates being divided into five groups. In table 1 is given the sexual progeny mean for each segregate and the distribution of the segregates in each generation among the five breeding types.

A comparison of the five breeding types is made in table 2, where the individual progenies of each type are combined and the distribution of the individuals from the progenies of each type among the classes of scab rating is shown. The distribution for each type is significantly different from that of the other types. This is indicated also by the differences between the type means and by the probability from χ^2 tests which is less than 0.01 in all cases.

This study, as previously stated, was undertaken on the hypothesis that a gene was present which had a major influence on scab reaction and that this would allow the classification of the breeding material into five genotypic classes. A comparison of the distribution of the segregates for each generation of the cross among the five types of breeding behavior with that of the distribution of the genotypes for each generation calculated on the assumption that the differences between breeding types was principally due to a single gene, can be made.

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TABLE 1.—*Mean scab ratings of progenies of* F_2 *and* F_3 *segregates of a cross between Lookout Mountain and Accession 123* [1]

F_1 segregates		F_2 segregates		F_3 segregates		Breeding type
Designation of segregate	Progeny mean	Designation of segregate	Progeny mean	Designation of segregate	Progeny mean	
--		5-3-1	0.67		------	1
5-3	1.17	5-14-6	1.00	5-4-14-1	1.17	2
5-13	1.17	5-9-1	1.10	5-12-1-1	1.20	
--	-	5-8-2	1.10	5-6-1-1	1.22	
-		5-2-1	1.11		---	
-	--	5-10-3	1.19		- -	
5-14	1.31	5-14-3	1.27	5-15-1-3	1.35	3
5-16	1.32	5-10-1	1.27	5-10-1-1	1.37	
5-12	1.33	5-14-15	1.30	5-4-3-6	1.38	
5-10	1.40	5-7-2	1.33	5-10-1-2	1.47	
5-8	1.42	5-12-1	1.33	5-10-3-2	1.48	
5-9	1.42	5-8-1	1.36	5-10-1-3	1.55	
5-7	1.49	5-14-16	1.38	5-11-1-3	1.59	
5-1	1.50	5-2-5	1.40	5-14-5-1	1.63	
5-5	1.50	5-11-1	1.40	5-11-1-4	1.80	
5-11	1.76	5-15-1	1.50	5-10-1-5	1.89	
--		5-3-2	1.63			
		5-6-1	1.65			
		5-14-8	1.68			
		5-14-1	1.74			
		5-14-17	1.77			
		5-2-4	1.80			
		5-2-2	1.80			
		5-14-2	1.81			
-		5-10-2	1.82			
-		5-4-3	1.85			
		5-14-7	1.87			
5-4	1.92	5-4-1	2.05	5-14-8-1	1.96	4
5-2	1.98	5-6-2	2.21	5-4-1-1	2.00	
--		5-14-5	2.27	5-4-3-4	2.21	
		5-13-1	2.29			
		5-1-1	2.35			
			--	5-4-3-5	2.45	
				5-10-1-4	2.47	

[1] 2×S. E of a difference=0.38.

TABLE 2.—*Distribution of the individuals of the progenies of each breeding type*

Breeding type	Number of progenies	Number of individuals in the progenies of each type falling into the indicated classes of scab rating [1]					Mean	P from χ^2 test between types
		0	1	2	3	4		
	1	6	8	1	0	0	0.67	
	10	18	173	44	4	0	1.13	1 and 2 less than 0.01.
	42	46	598	377	105	19	1.52	2 and 3 less than 0.01
	11	8	77	125	94	17	2.11	3 and 4 less than 0.01
	2	2	15	13	19	15	2.47	4 and 5 less than 0.01

[1] 0=No scab, 4=most severe scab.

The 14 F_1 segregates are distributed among breeding types 2, 3, and 4. If the 5 types are represented by the following genotypes Sc_4, Sc_3sc, Sc_2sc_2, $Scsc_3$, and sc_4, then in the F_1 2 segregates would be classed as triplex, 10 as duplex, and 2 as simplex. A similar distribution in the F_1 might be obtained if 1 parent, Accession 123, was triplex and the other parent, Lookout Mountain, was simplex for the gene *Sc*. A comparison of the obtained with the calculated distribution is shown in table 3.

This assumption agrees with the information available concerning the parents, which unfortunately is meager since both were discarded before the scab studies were undertaken. Hybrids obtained from other progenies with Lookout Mountain have given progenies with very little resistance. On the other hand, selfed lines derived from Accession 123 have shown high resistance, some of them approaching that of Hindenburg selfed. Accession 123 was not homozygous since there was a significant difference in scab reaction between some of the selfed lines derived from it.

TABLE 3.—*A comparison of the obtained with the calculated genotype distribution in the F_1 segregates of the cross Accession 123 × Lookout Mountain*

Distribution—	Sc_4	Sc_3sc	Sc_2sc_2	$Scsc_3$	sc_4
Obtained	0	2	10.0	2	0
Calculated	0	3.5	7.0	3.5	0

The theoretical distribution which would be obtained in the F_2 and F_3 can be calculated on the basis that a random sample was taken from each generation. The segregates probably represented a random sample, for they included all of the self-fruitful plants in each generation. Unfortunately only a selected group was carried into each succeeding generation. However, the breeding plots were relatively free of scab, and no consideration was given to scab reaction in selecting the segregates for the succeeding generation. In table 4 the distribution is calculated on a chromosome rather than a chromatid type of segregation as the former is in closer agreement with the obtained distribution.

TABLE 4.—*A comparison of the obtained with the calculated genotype distribution in the F_2 and F_3 segregates of the cross Accession 123 × Lookout Mountain*

[Calculations made on a chromosome rather than a chromatid type of segregation]

Distribution of—	Sc_4	Sc_3sc	Sc_2sc_2	$Scsc_3$	sc_4
F_2 segregates					
Obtained	1	5	21	5	0
Calculated	1.2	7.2	15.2	7.2	1.2
F_3 segregates					
Obtained	0	3	10	3	2
Calculated	2	4	6	4	2

REACTION OF PROGENIES FROM OTHER VARIETIES AND SELECTIONS

The reaction to common scab of selfed progenies from 118 other varieties and selections have been studied. Tests were made in the field in 1936, 1937, 1938, and 1939 and in the greenhouse during the fall and winter of 1938–39. Since the level of infection varied in the different tests, each comparable group is listed separately in table 5.

These varieties and selections have been classified into five breeding types. In making this classification consideration has been given to: (1) Regions of discontinuity in the progeny means; (2) the reaction of progenies which were tested under more than one level of infection; (3) the reaction of the self lines from a variety or selection.

It will be noted in table 5 that the variety Hindenburg is the sole individual classified in breeding type 1. Its sexual progeny mean in the four trials in which it was tested was consistently low. This variety does not appear to be homozygous for all factors which influence scab reaction since two of its segregates, 14–6 and 14–7, gave a significantly higher progeny mean in the two trials in 1939.

TABLE 5 —*Mean scab ratings of selfed progenies from varieties and selections 1936–39*

1936		1937		1937		1938		1939			Breeding type
Variety or selection	Progeny mean[1]	Variety or selection	Progeny mean[2]	Variety or selection	Progeny mean[1]	Variety or selection	Progeny mean[1]	Variety or selection	Progeny means		
									Greenhouse[3]	Field[1]	
Hindenburg	1 10	Hindenburg	1 29					Hindenburg	1 19	1 74	1
11-1-25	1 00	11 1 25	1 67					HC15	1 21	2 13	2
1-1	1 07	1 1	1 79					Jubel	1 26	2 33	
1-4-1	1 37	1 4 1	1 81					5-10-1-29	1 27	2 19	
15-4	1 19							5-2-8	1 40	2 27	
82-11	1 16	82-11	2 05	22 1	1 43	11-1-2-2-4-3	1 89	5-15-1-4-1-4	1 33	2 64	3
11-8-1-9-4	1 22	11 8-1 9-4	2 19	3-4	1 46	11-1-2-2-4 2	1 94	124 35-2	1 62	2 60	
11-1-2-2-4	1 21	11 1-2-2-4	2 22	17 2	1 59	5-4 1	2 07	14-7	1 62	2 93	
5-10-1	1 21	5-10-1	2 52	11-9-1-8 1	1 70	1-1-6	2 11	5-10-1	1 30	3 15	
21-2-2	1 12	21-2-2	2 55	4 9-3-1 1-1-1 1	1 78	5-14 8 1 1	2 11	5-14-8 1-1	1 21	2 75	
29-13-5	1 22			4-11-1 3 3	1 87	12 2-4-1-7-3	2 13	41 1 1-7-6-1	1 62	3 06	
11-8 1-9-3	1 27			5-15-1-4-1 2	1 85	5-15-1-4 1 2	1 67	14-6	1 65	2 40	
11-1 2-1-1	1 29					15-2	2 22	5 10 3-23-4	1 69	2 81	
						5-10 1-19	2 25	15-2	2 15	2 81	
						5 10-1-17	2 58	11-6-1-1-1-1-1	1 78	2 96	
						5 14 8-1-2	2 58	17-2-2	1 80	3 12	
						16 1	2 59	17-2-1	1 84	2 50	
						4 25-6	2 63	165 35-1	1 86	2 37	
						11 8-1-9-4	2 66	5-10-3-4	2 00	2 20	
						15-1 2	2 69	5-10-3-14	1 86	2 19	
								5-4-1-1-7	2 10	2 50	
82-8	1 35			11-8	2 17	41 1-1 7 5-1	2 72	66-6-2	1 45	3 26	4
82-6	1 35			5 15-1-4-1	2 24	11-1 2 2 4 1	2 77	9-1-2	1 74	3 16	
19 33-1	1 36			30-11 3-1	2 36	15-1-1	2 93	5-4-1-1-3	1 82	2 73	
10-4-2	1 36					39-3-1 1-1 1	3 03	11-8-1-9-1 4	1 84	3 35	
5 33-1	1 38										
40-2-2	1 39					41 2-10 1	3 06	11-8-1-9-3-1	1 84	3 06	
82-4	1 40					82-1-1	3 07	66-6-3	1 97	2 80	
12-2-4-1-7	1 43	12-2-4-1-7	2 63					66-5-1	1 97	3 06	
82-10	1 44							Earlaine	2 00	2 69	
11-8-1-9-1	1 45							5-14-8-18	2 06	2 85	
11-1 2-1	1 51							5-10-3-23-2	2 19	3 04	
1-4-2	1 54							5-10-1-42	2 60	2 69	
34-1-1	1 62					34 1 1 1	3 09	124 35-1	2 00	3 34	
82 2	1 63					27 1	3 32	66-69-1	1 78	3 52	
82-1	1 64					82-4 1	3 33	11-1 2 3-2	1 86	3 40	
3-2-4	1 66					4 25-7-1	3 38	80-7-5	2 16	3 40	

29 14	1 80				13-1	3 46	Chippewa.	2 30	3 30	
81-1	2 00				21-2-2-11	3 50	80-7-1	2 37	2 87	
29-13	2 03				5-14-8-1-7	3 53	60-6-1	2 39		
11-1-3-2	2 10	11-1-3-2	2 91		13 1-1	3 60	66-4-1	2 45	3 22	5
-					11-1-3 2 18	3 67	5-4-1-1-4	2 70	3 73	
					80-11 3	3 70	80-7-6	2 71	3 53	
					11-1-3-2-11	3 70	80-7-3	2 75	3 06	
					11 1-25-3	3 71	80-7-2	2 79	3 42	
					11-1-3-2-14	3 82	80-7-4	2 88	3 57	
					11-1-3-2 20	4 00				
2 × S E of difference	0 43		0 33	0 38		0 46		0 35	0 54	

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[1] First clonal generation

[2] Second clonal generation

[3] Seedling generation.

In breeding type 2 are eight individuals, including the variety Jubel. The clonal reaction of these eight individuals is very similar to that of Hindenburg. Four of these individuals, 11-1-25, 1-1, 1-4-1, and 15-1, produced sexual progenies whose mean scab reaction was not significantly different from that of the progeny of Hindenburg in 1936. In the second clonal generation in 1937 the sexual progenies of three of these individuals appeared to give a significantly higher scab reaction than that of the progeny of Hindenburg. The remaining four individuals of breeding type 2 gave sexual progenies with a small but consistently higher scab reaction in both the greenhouse and the field test than that of Hindenburg in 1939.

Forty-two clones are classified in breeding type 3. The position of this group was marked by including comparable sexual progenies of selection 5-10-1 in five of the six tests. The limits of the group were determined by separation in the regions of the greatest discontinuity in the distribution of the progeny means. The separation in 1937 is the same as that made for the segregates of the cross given in table 1 since these were included in the same test. The trial in 1938 did not include the progeny of selection 5-10-1. However, this trial included the progeny of selection 5-15-1-4-1-2, of which a comparable sexual progeny was tested in 1937, and the progeny of selection 15-2, from which a similar sexual progeny was tested in the greenhouse and in the field in 1939. The sexual progenies of individuals classified in breeding type 3 appeared to be more variable than those from the other breeding types. Thus the sexual progeny of selection 5-10-1 gave a scab reaction similar to that of the individuals placed in breeding type 2 in 1936. Under a higher level of infection in the second clonal generation tested in 1937, this sexual progeny approached breeding type 4. Again in 1939 in the greenhouse test the progeny mean was relatively low, approaching that of the sexual progeny of the variety Jubel, and was significantly lower than the progeny of the variety Earlaine. In the field tests which followed a higher level of infection was obtained, and the progeny mean of selection 5-10-1 is significantly higher than that of the progeny of Hindenburg and Jubel and approaches the progeny mean of Earlaine. This indicated difference in reaction to high and low levels of infection in the tests has been considered in the classification of the individuals into breeding type 3. A similar difference in reaction to low and high levels of infection has been noted in the clonal reaction of this group. Their clonal resistance to scab is sufficient to be of commercial value under ordinary levels of infection.

In breeding type 4 are grouped 29 clones including the variety Earlaine. The variety Katahdin also probably belongs to this group since selections from its selfed progenies (line 66) are included. This group differs from breeding type 3 in having slightly higher sexual progeny means. However, their progeny means are significantly lower in general than those of breeding type 5. The latter are considered to carry no resistance. In clonal tests, individuals in breeding type 4 were distinguishable from those of type 3 but not from those of type 5. While more intensive studies will probably indicate the presence of a greater clonal resistance in the individuals belonging to breeding type 4 than those of type 5, it would hardly be sufficient to be of commercial value.

Thirty-five clones including the variety Chippewa are grouped in breeding type 5. This group shows no clonal resistance to scab. Attempts to isolate resistant individuals from their sexual progenies have met with no success. A few of the more promising isolates were given progeny tests. Thus it will be noted that the progenies of 11 1–3–2–(11) (14) (18) and (20), all selections from 11 1-3–2, gave high progeny means. Similar selections from line 80 7 gave high progeny means as will be seen from the progeny means of selections 80 7 (1) (2) (3) (4) (5) and (6).

REACTIONS OBTAINED IN CROSSING PARENTAL TYPES

The reaction to scab of the crossed progenies as obtained in the field in 1936 and 1938 and in the greenhouse and field in 1939 are presented in table 6. The parents employed were previously classified from the reaction of their selfed progenies with the exception of the varieties Early Ohio, Triumph, and Warba. The crosses are listed in the order of their mean scab reaction.

In 1936, crosses between parents of breeding types 2, 3, 4, and 5 were tested. The two crosses of type 2 (Jubel) by selections of type 3 and 4 gave the lowest progeny mean. The two crosses between parents of type 5 gave the highest progeny mean. The breeding behavior of the varieties Early Ohio, Triumph, and Warba appears to be the same. From the progeny means of the crosses in which these varieties enter, they are classified as probably belonging to breeding type 5.

Crosses between the same breeding types as were tested in 1936 were tested in 1938. The progeny means of these crosses are in fair agreement with the classification made from their selfed progenies with the possible exception of selection 80 7. This parent was classified in breeding type 5 on the basis of the high mean scab reaction of its selfed lines. Its behavior in these crosses suggests that it may carry some factors which influence low scab reaction.

The results with the crosses tested in the greenhouse and field in 1939 emphasize the low progeny means obtained when Hindenburg enters into a cross. The cross of type 2, (Jubel) by type 3, selection 5-10–1, gave a somewhat higher scab reaction than the average of the four crosses in which Hindenburg was crossed with selections of type 4. In the 1936 and 1938 tests, which contained no crosses with Hindenburg, the same cross, Jubel with 5 10 -1, gave the lowest average mean scab reaction. Clark et al.[8] from observations on two F_1 progenies concluded that Hindenburg was probably homozygous for resistance. The mean scab reaction of the selfed progeny of Hindenburg given in table 5 and its behavior in the crosses as shown in table 6 suggest that this variety is nearly homozygous for scab resistance. However, two selections, 14-6 and 14 7, from the selfed progeny of Hindenburg, produced selfed progenies with a significantly higher mean scab reaction than their parent progeny (see table 5). This suggests that it is heterozygous for at least some minor factors which influence the mean scab reaction. The results of the 1939 test also supply further evidence that the varieties Triumph and Early Ohio probably carry no factors influencing low scab reaction.

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[8] CLARK, F C, STEVENSON, F. J, and SCHAAL, L A. THE INHERITANCE OF SCAB RESISTANCE IN CERTAIN CROSSES AND SELFED LINES OF POTATOES Phytopathology 28 878–890, illus 1938

TABLE 6.—*Mean scab ratings of hybrid progenies from certain breeding types as determined in the field in 1936 and 1938 and in the field and greenhouse in 1939*

Parent	Breeding type	Parent	Breeding type	Number of seedlings	Progeny mean	
					Greenhouse	Field
		1936 TEST				
Jubel	2	× 5-14-8-1	4	15		1.00
Jubel	2	× 5-10-1	3	15		1.07
4-9-3-1-1-1-1-1	3	× 82-10	4	44		1.21
4-9-3-1-1-1-1-1	3	× 5-10-1	3	68		1.26
Early Ohio	5	× 4-9-3-1-1-1-1-1	3	11		1.27
Triumph	5	× 5-10-1	3	104		1.39
Warba	5	× 5-10-1	3	79		1.40
11-1-2-2-4	3	× 21-2-2	3	31		1.42
4-25-6	3	× 5-10-1	3	26		1.42
82-10	4	× 12-2-4-1-7	4	109		1.42
Early Ohio	5	× 5-10-1	3	34		1.44
11-8-1-9-4	3	× 21-2-2	3	67		1.46
Warba	5	× 12-2-4-1-7	4	17		1.59
Early Ohio	5	× 12-2-4-1-7	4	23		1.74
Warba	5	× 11-1-3-2	5	32		2.00
Triumph	5	× 4-25-7-1	5	26		2.38
2 × S. E. of difference						.43
		1938 TEST				
Jubel	2	× 5-10-1	3	47		2.08
30-11-3-1	4	× 5-10-1-29	2	22		2.73
17-2	4	× 5-10-1	3	69		2.85
11-8-1-9-3	3	× 80-7	5	22		2.95
82-11	4	× 80-7	5	37		2.97
17-3	3	× 5-10-1	3	51		3.01
15-2	3	× 80-7	5	72		3.04
80-7	5	× 5-10-1	3	61		3.08
80-7	5	× 15-2	3	51		3.18
13-1	5	× 80-7	5	36		3.25
11-8-1-9-3	3	× 5-10-1	3	21		3.31
21-2-2	3	× 5-10-1	3	44		3.35
15-2	3	× 5-10-1	3	17		3.35
82-6	4	× 15-2	3	30		3.37
82-6	4	× 5-10-1	3	47		3.38
13-1	5	× 5-10-1	3	43		3.51
11-1-3-2	5	× 5-10-1	3	28		3.52
13-1	5	× 11-1-3-2	5	28		3.75
2 × S. E. of difference						.46
		1939 TEST				
39-3-1-1-1-1	4	× Hindenburg	1	75	1.37	2.16
Hindenburg	1	× 5-14-8-1	4	69	1.65	2.21
41-1-1-7-5	4	× Hindenburg	1	43	1.44	2.40
Jubel	2	× 5-10-1	3	40	1.38	2.42
5-10-3-4	3	× 15-2	3	70	1.46	2.46
Triumph	5	× Hindenburg	1	42	1.08	2.53
82-8	4	× Hindenburg	1	90	1.38	2.67
5-15-1-4-1-2	3	× 11-1-25	2	88	1.75	2.72
HC 15	2	× 5-14-8-1	4	93	2.37	2.85
13-1	5	× Hindenburg	1	84	1.51	2.86
13-1	5	× 5-10-1	3	67	1.36	2.92
Triumph	5	× Jubel	2	70	1.39	2.95
11-1-3-2-18	5	× 5-10-1	3	10	2.14	3.00
Early Ohio	5	× 5-14-8-1	4	37	2.05	3.50
Triumph	5	× 5-14-8-1	4	70	2.30	3.39
5-10-3-4	3	× 11-1-3-2-18	5	54	2.68	
2 × S. E. of difference					.35	.54

ASSOCIATION BETWEEN THE *P* FACTOR AND RESISTANCE TO SCAB

The possible association between the plant color factor P and scab resistance was studied in 13 crosses. A susceptible P_{p_3} clone was crossed with the p_4 clone 5-10-1, which is classified as of breeding type 3. The P_{p_3} plants from the F_1 were then crossed with p_4 plants. The results are presented in table 7. The separation of the P and p plants is easy and accurate. By having the red color factors, either E or R, present, the plants with p have red and the plants with P have purple tubers. It will be noted that the plants with purple tubers (P) had a higher mean scab rating in all the crosses except one. The differences in mean scab rating are too small to be significant except in a cross of Russet Rural by 5-10-29. When all the crosses are considered the differences become highly significant.

TABLE 7.—*Mean scab ratings of P and p plants in 13 crosses*

Parents of cross		Plants with purple tubers (P)		Plants with red tubers (p)	
P_{p_3}	p_4	Number	Mean scab rating	Number	Mean scab rating
76-1	× 5-10-29	7	2.00	17	1.47
77-8	× 5-10-29	18	1.78	15	1.07
77-9	× 5-10-29	42	2.62	37	1.95
76-1	× 11-1	14	2.05	9	2.00
77-8	× 11-1	9	1.89	11	2.00
77-9	× 11-1	21	2.42	30	2.39
76-1	× 12-2	20	3.21	6	1.50
77-8	× 12-2	15	2.40	11	1.04
77-9	× 12-2	17	2.59	13	2.48
77-8	× 75-5	30	1.80	30	1.73
Russet Rural	× 5-10-29	15	2.73	27	1.93
Do	× 5-10-1	29	3.50	48	2.97
Do	× 5-14-8-1	14	3.36	6	2.80
Total or mean of 13 families		251	2.49	260	1.99

DISCUSSION

The autotetrasomic type of inheritance in the potato limited the study of classifying the varieties and selections to one based on the hypothesis that the reaction to scab was influenced sufficiently by one gene to allow a grouping of the material into the five possible genotypes. In classifying the segregates of the cross between Accession 123 and Lookout Mountain, the principal reliance was placed on the means of their sexual progenies. For in interpreting the results of progeny tests, it is necessary to remember that scab is a disease caused by a pathogen comprising many physiologic races and that the extent of infection is influenced by factors such as temperature, moisture, pH, and the stage of maturity of the plant. The use of the type of pustule, rather than the extent of scabbed surface, reduces the variability caused by these factors to some extent, but the variation is still too large to utilize a phenotypic classification of the individuals within the progenies.

Assuming that at least five genetic groups were present, the segregates of the cross between Accession 123 and Lookout Mountain were separated in the regions of the greatest discontinuity of their sexual progeny means into five groups designated as breeding types. The distribution of individuals within the sexual progenies of each breeding type was found by the χ^2 test to be significantly different from any of the other types.

The five breeding types were assumed to represent the following genotypes Sc_4, Sc_3sc, Sc_2sc_2, $Scsc_3$, and sc_4; and the parent genotypes were assumed to be, for Accession 123, Sc_3sc, and for Lookout Mountain $Scsc_3$. The observed distribution in the F_1, F_2, and F_3 was found to be in fair agreement with that calculated from the above hypothesis. This study appeared to justify an attempt to classify the breeding material from the scab reaction of their sexual progeny into breeding types. In making this classification, consideration was given to regions of discontinuity in the progeny means, to reactions of sexual progenies tested under more than one level of infection, and to the reaction of the sexual progeny of the segregates from a selfed line of a variety or selection. Further studies will undoubtedly suggest shifting some varieties and selections to groups different from those in which they are now located. These groups are not considered to be homogeneous, for the reactions to scab obtained from isolates of the sexual progenies of individuals classified in breeding types 1 and 5 indicate that these individuals are probably not homozygous for all factors influencing scab reaction. The results obtained from crosses between types agree reasonably well with the reactions to scab of the selfed progenies. The data indicate that testing varieties and selections by crossing would be of value when large differences are present. The data also supplied information on the breeding behavior of the Early Ohio, Triumph, and Warba varieties from which selfed seed was not available. The association obtained between the P factor and reaction to scab suggests that a major factor is present in this chromosome group, for as the experiment was set up, only the influence of a gene or possibly a number of genes in the simplex condition was measured.

SUMMARY

Starting with the assumption that the type of inheritance in the potato was autotetrasomic and that the difference observed in reaction to common scab was principally due to the influence of one gene, the segregates obtained from the F_1, F_2, and F_3 generations of a cross between Accession 123 and Lookout Mountain were classified into five breeding types. The separation was made in regions where the mean scab reaction of the sexual progenies showed the greatest discontinuity. The distribution of the segregates of the five breeding types in the F_1, F_2, and F_3 generation approached that calculated on the hypothesis that Accession 123 was triplex and Lookout Mountain was simplex for a gene Sc influencing reaction to scab and that the five breeding types corresponded to the genotypes Sc_4, Sc_3sc, Sc_2sc_2, $Scsc_3$, and sc_4.

One hundred and eighteen varieties and selections of heterogeneous origin were classified from sexual progeny tests into five groups corresponding to the above breeding types as follows:

Type 1. The variety Hindenburg.
Type 2. Seven selections and the variety Jubel.
Type 3. Forty-two selections.
Type 4. Thirty-one selections and the variety Earlaine.
Type 5. Thirty-four selections and the Chippewa variety.

Hindenburg, which was classified in type 1, gave a progeny from which two segregates were isolated whose sexual progeny gave a significantly higher mean scab reaction than the progeny of Hindenburg.

Crosses between types gave progenies whose mean reaction to scab was in general agreement with the reaction of the selfed progenies of the parents. The Early Ohio, Triumph, and Warba were classified in type 5, from the mean scab reaction of the crossed progenies, obtained when these varieties were used as female parents.

An association between the color factor *P* and the mean scab reaction was observed in a study of 13 crosses. The mean scab reaction of the *P* and *p* plants for the 13 crosses was 2.49 and 1.99 respectively.

USE OF THE PENETROMETER FOR DETERMINING THE FIRMNESS OF FATTY TISSUE OF HOG CARCASSES[1]

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INTRODUCTION

An accurate, moderately rapid, readily applied, and inexpensive method for determining the firmness of fatty tissue of chilled meat-animal carcasses would be of value to research workers concerned with the quality of meat, especially pork, in which there is much variation in firmness. Furthermore, it is possible that such a method would be useful under commercial conditions.

Several methods have been used for determining the firmness of animal fats or for determining the factors closely related to firmness. Among them are the manual test involving human judgment, the refractive index, iodine number, and the melting point. The manual test, as developed and applied by the Bureau of Animal Industry to the fatty tissue of hog carcasses, provides for the use of five degrees, or grades, of firmness—hard, medium hard, medium soft, soft, and oily. The chief objection to this method is the error introduced by the human factor. The error may be reduced considerably by having a committee of qualified judges. This is customary in research but is seldom done in commercial work. It has been shown[3] that a close relation exists between grade of firmness and refractive index, as well as between the former and iodine number. In the case of melting point, the relation was found to be less close. Other analyses have substantiated these findings. Values for 200 samples, representing 40 each of the 5 grades of firmness, gave the following coefficients of correlation:[4] Firmness grade and refractive index, -0.900 ± 0.004; firmness grade and iodine number, -0.899 ± 0.009; firmness grade and melting point, $+0.788 \pm 0.018$; refractive index and iodine number, $+0.960 \pm 0.004$.

Since there is little difference between refractive index and iodine number as a measure of firmness, the former has been extensively used by the Bureau as a routine laboratory test because of the greater rapidity and economy with which it can be determined. However, this method, as well as the iodine-number and melting-point tests, requires relatively expensive laboratory facilities and is rather time

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[1] Received for publication January 21, 1941. This work was conducted in cooperation with the Animal Nutrition Division of the Bureau.

[2] Acknowledgment is made to Mrs. Edna V. Steely for assistance in the statistical calculations.

[3] HANKINS, O. G., and ELLIS, N. R. SOME RESULTS OF SOFT-PORK INVESTIGATIONS. U. S. Dept. Agr. Bul. 1407, 68 pp., illus. 1926.

—— ELLIS, N. R., and ZELLER, J. H. SOME RESULTS OF SOFT-PORK INVESTIGATIONS, II. U. S. Dept. Agr. Bul. 1492, 50 pp., illus. 1928.

[4] The refractive indexes, iodine numbers, and melting points of these samples were determined by N. R. Ellis of this Bureau.

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consuming. Consequently an investigation of the possible merit of an instrument known as a penetrometer for measuring the firmness of chilled fatty tissue was believed to be desirable. This instrument has been used by scientific and commercial agencies with considerable success to determine the firmness of certain foods, such as cakes and jellies, by measuring the depth that a needle of specific size and bluntness will penetrate under a standard load during a specific time. It was felt that the manual test for firmness and the penetrometer determination involve the same principle. The objects of the study reported in this paper were to determine the usefulness of the penetrometer for measuring the firmness of fatty tissue of chilled hog carcasses and to establish tentative grade limits in terms of penetrometer readings in order that individual carcasses may be classified with respect to firmness.

EXPERIMENTAL PROCEDURE

Fat samples from the carcasses of 188 hogs slaughtered in the spring and fall of 1938 and the spring of 1939 in various projects conducted at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., were used in the present experiment. After being chilled for approximately 72 hours at a temperature of 33° to 35° F. (0.56° to 1.67° C.), the carcasses were graded for firmness by a committee of three qualified judges. The designated grades were hard, medium hard, medium soft, soft, and oily. The range in firmness of the carcasses was too narrow to provide good representation for all 5 grades. One of the writers, therefore, visited a commercial packing house in the spring of 1939 and selected 163 carcasses from which supplementary samples were obtained. He graded the carcasses and, on returning to Beltsville, graded the 163 samples with the assistance of 2 other experienced men. From the 2 sources mentioned, samples were procured from carcasses graded as follows: 116, hard; 70, medium hard; 49, medium soft; 65, soft; and 51, oily.

In each instance the sample of fatty tissue was removed from the carcass along the middle of the back according to the method described by Hankins and Ellis.[5] The skin was then removed, and the sample was squared and trimmed to the proper length to fit the sample box, which had inside dimensions of 3 by 3 by 1 inches. Immediately prior to testing, a thin slice was removed from the median side of the fat sample in order to provide a fresh, undried surface, and the sample was placed in the box with the surface upward. Figure 1 shows a sample ready to be placed in the box and another sample in the box ready for testing. No cover or weight was used to hold the sample in place; to keep it from slipping sideways in the box, pieces of fatty tissue were packed around it. Care was exercised not to put, on the sides of the sample, any pressure that might influence the depth of penetration of the needle.

The penetrometer needle used was 3 inches long with the working end 0.15 inch in diameter. The rounded point represented a radius of 0.075 inch. The total weight of the needle, the test rod that held it, and added load was 255.65 gm. With the aid of the mirror attached to the penetrometer the technician adjusted the working unit, consisting of the needle, test rod, and added load, so that the point of the needle barely came in contact with the sample. The weight of 255.65 gm. was then allowed to force the needle into the sample for 15 seconds. Three penetration tests were made on the outer layer of back fat and three tests on the inner layer, and the average of the six determinations was recorded as the value for the sample as a whole. Penetration was determined in tenths of a millimeter and at a sample and laboratory temperature within the range of 33° to 35° F. (0.56° to 1.67° C.)

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[5] HANKINS, O G, and ELLIS, N R. See footnote 3

FIGURE 1.—Penetrometer used in making determinations on the fatty tissue. A sample is being tested, with another sample, at the left, ready for testing.

After the penetrometer tests had been made the samples were rendered at 230° to 239° F. (110° to 115° C.) and filtered. Refractive-index values of the fat were then determined at 104° F. (40° C.)[6]

USEFULNESS OF PENETROMETER FOR DETERMINING FIRMNESS

It is shown in table 1 that as firmness decreased from hard to oily the depth of penetration of the needle increased for both the outer and inner layers of back fat. On an average, for the two layers there was a difference of 60.3 units, or 6.03 mm., between the greatest and least depths of penetration. For the hard and medium-hard grades there was slightly greater penetration into the outer layer than into the inner layer of back fat. On the other hand, for the medium-soft, soft, and oily grades, the greater penetration was into the inner layer.

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[6] The refractive-index values for study in relation to penetrometer determinations and firmness grades were determined by Willard E. Folland of the Bureau.

TABLE 1.—*Mean, range, and standard deviation of penetrometer determinations*[1] *and refractive indexes of fat samples representing five degrees of firmness*

Committee firmness grade (manual test)	Number of samples	Penetrometer determinations, in tenths of a millimeter					Refractive index		
		Outer layer of back fat	Inner layer of back fat	Average of outer and inner layers of back fat	Range[2]	Standard deviation[2]	Average of outer and inner layers of back fat	Range[2]	Standard deviation[2]
Hard	116	20.8	16.4	18.6	10–42	5.3	1.4595	1.4589 1.4602	0.0003
Medium hard	70	27.6	24.6	26.1	11–45	8.1	1.4601	1.4592 1.4611	.0004
Medium soft	49	37.9	44.9	41.4	19–59	9.6	1.4607	1.4595 1.4615	.0005
Soft	65	46.4	74.0	60.2	29 96	14.4	1.4615	1.4598 1.4631	.0006
Oily	51	56.4	101.4	78.9	48–109	11.7	1.4622	1.4616 1.4632	.0003

[1] Conditions of experiment: Radius of point of needle, 0.075 inch, total weight of needle and added load, 255.65 gm.; period of penetration of needle, 15 seconds.

[2] Represents values for average of outer and inner layers of back fat.

Table 1 also shows for each firmness grade the range and standard deviation of the penetrometer determinations. Similar data are given for refractive indexes. Between the medium hard and hard grades, medium soft and medium hard, soft and medium soft, and oily and soft, respective differences of 7.5, 15.3, 18.8, and 18.7 units in depth of penetration were found. The corresponding differences in refractive index were 0.006, 0.006, 0.008, and 0.007.

Also it is shown in table 1 that within each of the five groups of samples, classified according to committee grade for firmness, considerable variation occurred in the depth to which the needle penetrated. Likewise, rather wide variation in refractive index within the grade is indicated. Figure 2 shows graphically the variation of penetrometer determinations within the grade. Standard deviations for the soft and oily grades are larger than those for the hard grades.

A calculation of coefficients of correlation for data obtained by a committee of judges using the manual test for firmness, the penetrometer determinations, and the refractive-index values showed the closest relation between the committee grade for firmness and refractive index, the coefficient being −0.917±0.006. The next highest coefficient was −0.905±0.007, representing the correlation between committee grade for firmness and penetrometer determination. The negligible difference between these relationships indicates that the refractive index or penetrometer determination may be used with equal satisfaction in measuring firmness. Between refractive index and penetrometer determination the correlation was +0.878±0.008. Figure 3 is a scatter diagram for this last-mentioned relationship and shows also the regression line.

As previously stated, committee grade is not an exact measure of firmness since some error due to the human factor is involved. More-

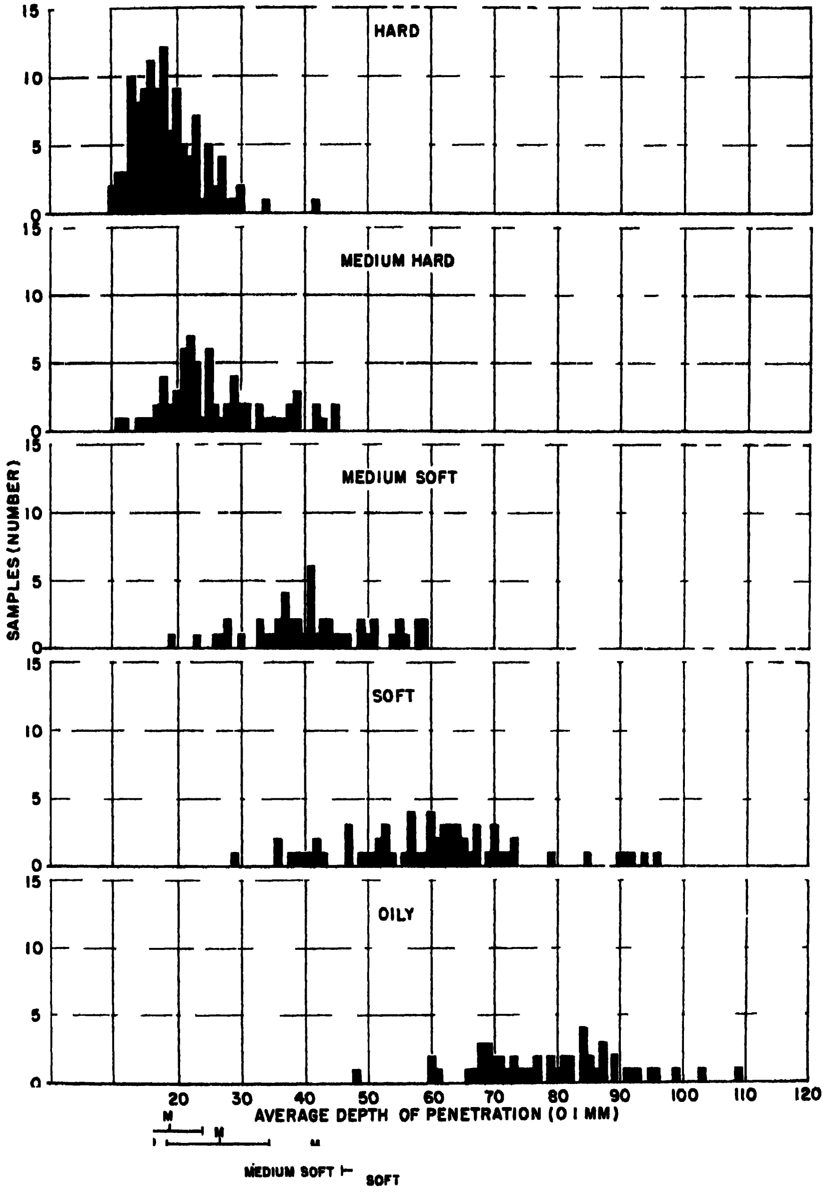

FIGURE 2 —Frequency distributions of firmness determinations made on hard, medium-hard, medium-soft, soft, and oily samples of fatty tissue, on the basis of depth of penetration by penetrometer For each distribution the mean (M) and standard deviation of the penetrometer determinations are shown

over, it is possible that the standards of firmness of the graders are subconsciously changed from time to time. In support of this point there are indications that in the work at Beltsville, during a period of 20 years, there has been a slight tendency to lower this standard of firmness. This is shown by comparison of the average refractive-index values in table 1 of this paper with those of Hankins and Ellis,[7] table 1. If there were no error in the committee grades and if the same principle were involved in the manual and penetrometer tests, the correlation between committee grade and penetrometer determination would approach 1 very closely.

ESTABLISHING TENTATIVE GRADE LIMITS IN TERMS OF PENETROMETER READINGS

With overlapping of grades, as indicated by penetrometer determinations or any other constant, and a lack of homogeneity of the variances[8] in the five groups, the investigator is confronted with a somewhat perplexing problem in attempting to designate firmness grade limits After considering several possible procedures in the study reported here, the writers reached the conclusion that the averages of penetrometer determinations for each of the five grades and the respective standard deviations should be used as a basis in establishing the tentative limits for these grades. After accepting 18.6 and 26.1 as means and 5.3 and 8.1 as standard deviations (table 1) for hard and medium-hard samples, respectively, the problem was to designate the most satisfactory maximum determination for the former grade and the minimum determination for the latter. It seems obvious from the foregoing that samples with penetrometer determinations 19 and 20 would be classified as hard, whereas those with determinations 25 and 24 would be classified as medium hard. Samples with intermediate determinations 21, 22, and 23 are as yet unclassified. Table 2 shows

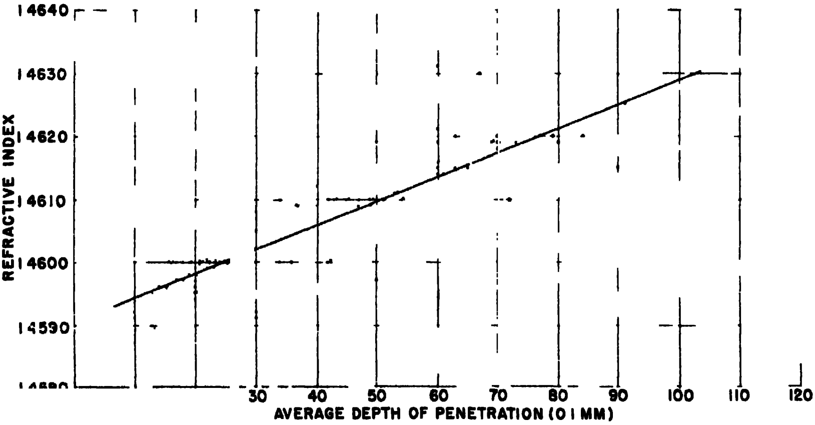

FIGURE 3 — Scatter diagram and regression line of refractive index and penetrometer determinations as indication of firmness of 351 samples of back fat

the deviations of each of these three determinations from the two means and the proportion of each deviation in terms of the corresponding standard deviation.

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[7] HANKINS, O G, and ELLIS, N R See footnote 5

[8] For the method employed see pp 206 207 of SNEDECOR, GEORGE W STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY Ed 3, 422 pp, illus 1940

TABLE 2.—*Deviations of penetrometer determinations* [1] *of 21, 22, and 23 from the means for hard and medium-hard grades and the proportion of each deviation in terms of the corresponding standard deviation*

Penetrometer determination [2] (0.1 mm.)	Deviation from mean for hard grade	Proportion of deviation in terms of standard deviation for hard grade	Deviation from mean for medium-hard grade	Proportion of deviation in terms of standard deviation for medium-hard grade
		Percent		*Percent*
21	2.4	45	5.1	64
22	3.4	64	4.1	51
23	4.4	83	3.1	38

[1] See footnote 1 of table 1.

[2] Represents values for average of outer and inner layers of back fat.

As shown by the table, in the case of the penetrometer determination 21 its deviation from the mean for hard samples is only 45 percent of the standard deviation of such samples. On the other hand, its deviation from the mean for medium-hard samples is 64 percent of the standard deviation for samples of that grade. In view of the greater probability of the value 21 falling within the standard deviation for the hard grade, one would be justified in assigning it to that grade rather than to medium hard. Further comparison of the percentages in the third and fifth columns of table 2 indicates that the determinations 22 and 23 should be included in the latter grade. Therefore, the penetrometer determination 21 is offered as the tentative upper limit of the hard grade, whereas 22 is offered as the tentative lower limit of the medium-hard grade.

With the same procedure, the upper limit for medium-hard grade and limits for the other grades were determined. The proposed limits for the five grades are as follows: Hard, 21 or less; medium hard, 22 to 33, inclusive; medium soft, 34 to 48; soft, 49 to 70; oily, 71 or more

SUMMARY AND CONCLUSIONS

Study was made of 351 back-fat samples from hog carcasses that had been chilled for 72 hours at 33° to 35° F. (0.56° to 1.67° C.) to determine the usefulness of a penetrometer for measuring their firmness. Another object was to establish tentative grade limits in terms of the penetrometer determinations for each of five degrees of firmness hard, medium hard, medium soft, soft, and oily. A penetrometer of standard type designed to determine firmness or consistency of foods and other materials was employed. The penetrometer needle was 3.0 inches long and 0.15 inch in diameter. The radius of the rounded, working point of the needle was 0.075 inch. A weight of 255.65 gm. and a time interval of 15 seconds were used. Penetration was determined in tenths of a millimeter. The study was conducted in 1938 and 1939 at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md.

The average penetrometer determinations for the back-fat samples, divided into the five degrees of firmness by a committee of three qualified judges, increased from 18.6 for hard to 78.9 for oily samples. This represents a difference of 60.3 units of measurement, or 6.03 mm. Average refractive-index readings for these samples also showed an increase—from 1.4595 for hard to 1.4622 for oily samples.

Correlation coefficients showed that a close relationship existed between (1) the committee grade for firmness and penetrometer determination, (2) committee grade for firmness and refractive index, and (3) penetrometer determination and refractive index.

For the several grades of firmness the following limits in terms of penetrometer determinations have been established tentatively: Hard, 0 to 21, inclusive; medium hard, 22 to 33; medium soft, 34 to 48; soft, 49 to 70; and oily, 71 or more.

It is obvious that the depth of penetration for any given degree or grade of firmness depends at least on the shape and size of the needle point, the weight of the working unit, and the period during which the needle is allowed to penetrate into the sample. By changing one or more of these three factors possibly more useful grade means and limits could be established. That is a problem for future study.

THE HEMICELLULOSES OF SUGARCANE FIBER (BAGASSE)[1]

By B. L. Davis, *junior chemist*, and Max Phillips, *senior chemist, Agricultural Chemical Research Division, Bureau of Agricultural Chemistry and Engineering, United States Department of Agriculture*

INTRODUCTION

Although that fraction of the carbohydrates of plants which Schulze (*13*)[2] designated as "hemicelluloses" has been investigated by chemists for about 50 years, the chemistry of this class of substances is still in a rather confused state. A brief review of the chemical literature on hemicelluloses has been given in two previous papers (*10*, *15*) published by this Bureau. For a more extensive critical résumé of the literature, the reader is referred to a book by Norman (*8*).

There is considerable information in the literature on the chemical composition (as determined by conventional methods) of sugarcane (*Saccharum officinarum*) fiber (bagasse). The composition of the hemicelluloses of this substance, however, has been the subject of only two chemical examinations. Prinsen Geerligs (*12*) isolated the hemicelluloses of bagasse in an impure state by extraction with cold 5-percent aqueous sodium-hydroxide solution and precipitation with acetic acid and alcohol. Upon hydrolysis with boiling 2-percent sulfuric acid, the dark gummy mass yielded a sugar that had a specific rotation of +20° and was undoubtedly xylose. In addition to this sugar, there were indications that in the hydrolysate there was probably present "another sugar with a higher rotatory power, perhaps arabinose or dextrose proceeding from the hydrolysation of cellulose."

Browne (*2*) digested sugarcane bagasse (which had been freed of sugar) for 3 hours in a boiling water bath with 5-percent aqueous sodium-hydroxide solution, concentrated the aklaline extract to one-third of its original volume, and precipitated the crude hemicelluloses with ethanol. This crude material was thoroughly ground up in a mortar with an excess of alcoholic hydrochloric acid, then was washed with 80-percent ethanol until free of acid, and finally was washed with 95-percent ethanol and ether. On hydrolysis with 4-percent hydrochloric acid, the product afforded *d*-xylose, together with a small quantity of *l*-arabinose.

The investigation reported in this paper was undertaken for the purpose of determining the character and composition of the hemicelluloses of sugarcane fiber (bagasse) by methods recently developed.

METHODS AND RESULTS

A quantity of sugarcane (variety Co. 281) from the field headquarters of the Bureau of Agricultural Chemistry and Engineering at the United States Sugar Plant Field Station, Houma, La., was crushed in a small laboratory mill, and the bagasse was cut into small pieces,

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[1] Received for publication February 26, 1941.

[2] Italic numbers in parentheses refer to Literature Cited, p. 246.

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dried in a steam drier, and then air-dried. A portion of this was ground in a Wiley mill fine enough to pass a 60-mesh sieve and dried in the oven at 105° C. The following constituents were determined (in percent):[3] Ash, 2.37; nitrogen, 0.24; uronic acids (as anhydrides), 4.1; pentosans, 22.06; methoxyl 2.46; lignin, 9.09; methoxyl in ash-free lignin, 15.9. (All results, except the percentage of ash, were calculated on the basis of oven-dried and ash-free material. The percentage of ash was calculated on the basis of oven-dried material.)

The bulk of the air-dried bagasse was extracted for 30 hours with a 1:2 alcohol-benzene solution in a continuous extraction apparatus. The extracted material was heated on the steam bath until free of solvent and then ground in a Wiley mill fine enough to pass a 60-mesh sieve. To 800 gm. of the extracted bagasse (795.2 gm. of moisture-free material), 8 liters of hot water (85° C.) was added. The mixture, stirred mechanically, was digested for 2 hours at 85° C. It was then filtered, and the residual material was again digested with hot water under the conditions just described except that the digestion period was increased to 16 hours. The aqueous extracts were combined, filtered, and concentrated under reduced pressure to approximately 1 liter. To the concentrated extract was added 3.5 volumes of 95 percent ethanol. The precipitate was filtered off, washed with graded strengths of ethanol,[4] and dried in vacuo over anhydrous calcium chloride. The precipitate weighed 10 gm.

The bagasse that had been extracted with hot water was treated with 10 times its weight of hot (85° C.) 0.5-percent aqueous ammonium oxalate solution, and the mixture was digested for 24 hours at 85° C. During part of this digestion period the reaction mixture was stirred mechanically. It was then filtered, and the filtrate obtained in this extraction operation, like that from the previous one, was concentrated under reduced pressure. To the concentrated solution 3.5 volumes of 95-percent ethanol, which had been acidified with hydrochloric acid, was added. The precipitate was separated with the aid of the centrifuge, washed with 75-percent ethanol until free of oxalate, and finally was washed with graded strengths of ethanol and with ether. It was dried in vacuo over phosphorus pentoxide in the Abderhalden drier at 56° C. The crude pectin thus obtained weight 7.5 gm.

PARTIAL DELIGNIFICATION

The bagasse that had been extracted with 0.5-percent ammonium oxalate solution was washed with cold water and then treated with 10 times its dry weight of an alcoholic sodium hydroxide solution (made by dissolving 20 gm. of sodium hydroxide in 150 cc. of water and 800 cc. of 95-percent ethanol). The mixture was digested at room temperature for 24 hours, during which it was stirred intermittently with a mechanical stirrer. The plant material was filtered off and digested twice with alcoholic sodium hydroxide, as previously described. The combined alkaline alcoholic extract was neutralized with hydrochloric acid, and the solution was concentrated at 50° C. under reduced pressure. The concentrated solution was acidified with hydrochloric acid, and the precipitated lignin was filtered off, washed with water until free of acid, and then dried in vacuo at 60° C.] The yield of lignin amounted to 33 gm. It was a yellow amorphous material. No furfural was obtained when this lignin was distilled with 12-percent hydrochloric acid.

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[3] Ash was determined by burning the sample in an electric muffle at 600° C. Nitrogen was determined by the Kjeldahl-Gunning Arnold method, and pentosans by the Tollens-Krober procedure, as described by the Association of Official Agricultural Chemists (*1*). The uronic acids were determined according to the procedure recommended by Dickson, Otterson, and Link (*3*), as modified slightly by Phillips, Goss, and Browne (*11*). The percentage of methoxyl was determined by the method described by Phillips (*9*). The methyl iodide was absorbed in pyridine. Lignin was determined by the method of Goss and Phillips (*4*).

[4] The term "graded strengths" of ethanol as used in this paper means 70-percent, 85-percent, 95-percent, and absolute ethanol.

The filtrate from the lignin precipitate was neutralized with sodium hydroxide solution and evaporated to dryness on the steam bath. When the residue was distilled with 12-percent hydrochloric acid no furfural was obtained, indicating that in all probability the method of delignification used did not result in the removal of any of the furfural-yielding constituents.

ISOLATION OF THE CRUDE HEMICELLULOSES

The residual bagasse from the delignification operation described above was heated on the steam bath until the alcohol had evaporated. It was then mixed with sufficient 5-percent aqueous sodium hydroxide solution to make a thin suspension, and the mixture was allowed to digest at room temperature for 24 hours. The reaction mixture was stirred from time to time. At the end of the 24-hour period, the mixture was filtered, and the digestion of the residual material with 5-percent aqueous sodium hydroxide solution was repeated three times. To the combined alkaline extracts 3 volumes of 95-percent ethanol was added, and the precipitated hemicelluloses were separated with the aid of the centrifuge. The product was washed with ethanol, to which a little acetic acid had been added, until free of alkali and was then washed with ethanol alone and finally with ether. The hemicellulose preparation was dried in vacuo over anhydrous calcium chloride, which freed it from most of the ethanol and ether. It was then moistened with an equal weight of water and chlorinated for 1 hour, the procedure described in a previous publication (*15*) being generally followed. The chlorinated product was mixed with enough 95-percent ethanol to form a thin suspension, the mixture was well shaken, and the alcohol was separated with the aid of the centrifuge. To the residual material a 3-percent ethanolamine solution in 95-percent ethanol was added, and the mixture was heated for 10 minutes under a reflux condenser in a water bath maintained at 80° C. The reaction mixture was allowed to cool to 50° C. and then was centrifuged. The supernatant liquid was drawn off, and the extraction with ethanolamine solution was repeated. The extracted hemicelluloses, after being washed with 95-percent ethanol, were suspended in a 5-percent aqueous sodium hydroxide solution and precipitated by the addition of three volumes of 95-percent ethanol. The hemicelluloses were separated with the aid of the centrifuge, washed with neutral 70-percent ethanol, then with 70-percent ethanol acidified with acetic acid, and again with neutral 70-percent ethanol. The material was then washed with graded strengths of ethanol and finally with anhydrous ether. It was dried first in a vacuum desiccator over anhydrous calcium chloride at room temperature and finally in a vacuum oven at 40° C. The yield amounted to 117 gm. An analysis of this product gave the following results (in percent): Ash, 1.58; nitrogen, 0.03; uronic acids (as anhydrides), 4.96; pentosans, 95.36; lignin, 0.20; methoxyl, 0.50. (All percentages except that for ash were calculated on the basis of moisture-free and ash-free material. The percentage of ash was calculated on moisture-free material.)

HYDROLYSIS

In a preliminary experiment the hemicellulose preparation (35.4 gm. of moisture-free and ash-free material) was treated with 2,000 cc. of 2.5 percent sulfuric acid, and the mixture was boiled under a reflux condenser for 15 hours. The reaction mixture was allowed to cool, and the dark insoluble material was filtered off. To the filtrate approximately nine-tenths of the calculated quantity of barium hydroxide solution was added slowly, while the reaction mixture was stirred mechanically and the temperature was maintained at 40° C. An excess of powdered barium carbonate was then added, and the mixture was heated at 70° C. until the acid was completely neutralized. Some decolorizing carbon (Norit) was then added, and the mixture was heated for another 15 minutes. It was then filtered, the insoluble material was washed with hot water, and the washings were added to the main filtrate. The filtrate was concentrated to a volume of approximately 150 cc. under reduced pressure at a temperature not exceeding 50° C. The concentrated solution was poured slowly, with stirring, into four volumes of absolute ethanol. The precipitated barium salt was separated with the aid of the centrifuge. After the clear supernatant liquid was drawn off, the barium salt was washed with absolute ethanol and with anhydrous ether and was then dried in vacuo over sulfuric acid. The dried material weighed 1.2 gm.

The filtrate from the barium salt was decolorized with carbon (Norit) and concentrated under reduced pressure at 50° C. to a thin sirup. This was poured, with stirring, into 4 volumes of absolute ethanol, and only a slight precipitate was produced. The alcoholic solution was centrifuged, and the clear supernatant liquor separated from the small quantity of gummy matter was again concentrated under reduced pressure at 50° C. The sirup was allowed to remain in a desiccator over anhydrous calcium chloride until crystals began to form. It was then allowed to remain in an electric refrigerator until a considerable quantity of sugar had crystallized out and the mixture was then filtered. The sugar was recrystallized from 75 percent ethanol, and after drying in the vacuum desiccator over sulfuric acid it weighed 16.0 gm. This sugar was identified as *d*-xylose by its melting point, by the refractive indices of its crystals,[5] and by Bertrand's method (*5*). Bertrand's double cadmium salt was also identified by determining the refractive indices of its crystals and comparing them with a known specimen of this salt.

From the sirup remaining after the separation of the *d*-xylose crystals, a diphenyl hydrazone was prepared. This was identified as the diphenyl hydrazone of *l*-arabinose by its melting point and by the optical properties of the crystals. On oxidation with nitric acid the sirup yielded oxalic acid but no mucic acid or saccharic acid. Both the Pinoff (*5*) test for fructose and the test for mannose were negative.

The barium salt obtained in the hydrolysis of the hemicellulose preparation described above gave Tollens' (*14*) naphthoresorcinol test for uronic acids. On oxidation with either nitric acid or bromine water, it gave no mucic acid, thus indicating the absence of galacturonic acid. The barium salt was dissolved in water, and the calculated quantity (less 5 percent) of standard sulfuric acid solution required to liberate the combined acid was added, and the mixture was warmed for 15 to 20 minutes at 60° C. The barium sulfate was then filtered off, and the filtrate was concentrated under reduced pressure to 50 cc. (the temperature of the bath during concentration did not exceed 45°). The concentrated solution was poured into 200 cc. of absolute ethanol, and the mixture was allowed to stand overnight, after which the excess barium uronate was filtered off. The alcoholic filtrate was distilled under reduced pressure at a temperature not exceeding 45°, and the concentrated solution was diluted with water to a volume of 40 cc. To this solution was added a quantity of solid cinchonine somewhat in excess of that required for complete neutralization of the acid, and the mixture was warmed on the steam bath for 15 minutes. The excess of cinchonine was filtered off, and the filtrate was extracted with two 20-cc. portions of chloroform. The aqueous solution was concentrated in the vacuum desiccator over calcium chloride. The cinchonine salt obtained was recrystallized from water. It melted at 190° C. (corrected). When mixed with some pure cinchonine glucuronate, the mixture melted at 189° (corrected). It was not possible to establish the identity of the salt by optical methods, because the refractive indices of the crystals of cinchonine glucuronate and of cinchonine galacturonate were found to be so nearly alike as to be indistinguishable.

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[5] All identifications by optical methods were made by G. L. Keenan of the Microanalytical Division of the Food and Drug Administration, Federal Security Agency.

The brucine salt was prepared in a similar manner. When the melting point of the salt was determined, the following was noted: At about 170° C. the material shrank away from the side of the melting-point tube, and at 193°-195° (corrected) it melted without any noticeable decomposition. The same behavior was noted when the melting point of a known and pure specimen of brucine glucuronate was determined. As in the case of the cinchonine salt, it was impossible to establish the identity of the salt by optical methods and for the same reason.

It cannot be said that the identity of the uronic acid was established beyond any doubt; nevertheless, the melting points of the two salts, together with the fact that no mucic acid was obtained on oxidation, are strong indications that the uronic acid obtained was glucuronic acid.

A second batch of hemicellulose, corresponding to 44.33 gm. of moisture-free and ash-free material, was hydrolyzed with 2,500 cc. of 2.5-percent sulfuric acid, following the procedure described above. After removal of the barium uronate, the alcohol was removed from the solution under reduced pressure at 50° C., after which the concentrated sugar solution was diluted with water to a volume of 500 cc. The total reducing sugars in this solution, as determined by the method of Munsen and Walker (*1*) amounted to 39.94 gm. (calculated as glucose). When a 3-cc. aliquot of the sugar solution was distilled with 12-percent hydrochloric acid, and the distillate was treated with phloroglucinol solution, following the procedure used for the determination of pentosans, 0.2366 gm. of phloroglucide precipitate was obtained. This is the equivalent of 20.92 gm. of furfural in the total solution. The sugar solution was also analyzed for arabinose by the Wise and Peterson (*16*) modification of the method of Neuberg and Wohlgemuth (*7*), and was found to contain a total of 1.40 gm. of arabinose. The percentage of furfural afforded by the hemicellulose preparation, when distilled with 12-percent hydrochloric acid, was also determined.

The analytical data and the composition of the hemicellulose preparation calculated from these data are as follows (in percent):[6] Uronic acid (as anhydride), 4.96; total furfural, 55.99; furfural from uronic acid, 0.94; *l*-arabinose, 3.70; furfural from *l*-arabinose, 1.77; furfural from *d*-xylose (by difference), 53.28; *d*-xylose (calculated from furfural), 92.5; molar ratio of uronic acid to *l*-arabinose and *d*-xylose, 1:0.87:21.9.

SUMMARY

The hemicelluloses were isolated from sugarcane fiber (bagasse) which had previously been freed of sugars and pectin. The product obtained was light gray and was practically free of nitrogenous substances and lignin. On hydrolysis with dilute sulfuric acid it afforded *d*-glucuronic acid, *l*-arabinose, and *d*-xylose in the approximate molar ratio of 1:0.87:21.9.

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[6] In calculating the yield of furfural from the percentage of uronic acid anhydride, the assumption was made that the uronic acid was *d*-glucuronic acid. According to Lefèvre and Tollens (*6*), *d*-glucuronic acid yields furfural in the proportion of 1 part of furfural to 5.25 parts of glucuronic acid anhydride. In estimating the furfural corresponding to the *l*-arabinose actually found, a correction was applied for the furfural destroyed during the hydrolysis. In calculating the percentages of furfural derived from *l*-arabinose and *d*-xylose, consideration was given to the fact that these sugars furnish 75 and 90 percent, respectively, of the theoretical yield of furfural.

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FACTORS INFLUENCING RATE OF GAIN OF BEEF CALVES DURING THE SUCKLING PERIOD[1]

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INTRODUCTION

The following study was undertaken as part of a broad project to evaluate the factors that influence the efficiency of feed utilization in cattle. One of the objects of a record-of-performance program with cattle is to devise a means of evaluating growth ability and efficiency of gain at an early age. Knapp, Lambert, and Black (*5*)[2] have shown that birth weight is of limited value as an index of a calf's growth potentialities. They showed that a calf's prenatal growth is primarily an expression of the dam, and therefore that little of the variation in prenatal growth can be attributed to genetic differences between calves.

The next period of development, the suckling period, is at first one of adjustment to a new environment following parturition. Milk makes up the larger portion of the diet, though in the latter part of the suckling period other feeds gradually replace it. Since milk is an excellent food for growth, the suckling period is one of large skeletal increase. Approximately 80 percent of the mature skeletal size of cattle has been attained by the end of the suckling period, whereas only about 40 percent of the mature weight has been reached. If it were possible to evaluate an animal by the end of the suckling period, a great amount of time and labor might be saved. The study reported in this paper was made to determine the factors that influence the rate of gain in calves during the suckling period and also to ascertain the value of rate of gain during this period as a basis of selection for breeding.

PREVIOUS INVESTIGATION

Donald (*3*), as a result of a study of milk consumption and growth of suckling pigs, stated:

> The effect of an investigation such as this is to invite reconsideration of the importance of variability in weaning or 3-week weight. Differences in size among young pigs are probably useless for comparing their genetic quality. Such differences as have been found, and even some deaths, can be satisfactorily explained by variations in the level of nutrition.

He also stated:

> The lightest pigs at or before weaning are by no means always the "poorest doers" in later life.

Donald (*4*) also made a comparison of pigs reared by foster mothers with those reared by their own dams. Half of each litter of pigs

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[2] Italic numbers in parentheses refer to Literature Cited, p. 254.

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was transferred to a foster mother and half remained with the natural dam. Thus half of the pigs suckled by each sow were foster pigs and half were her own. It was found that the milk production of the sow and other influences attributable to the sow had a greater effect on the gains of the animals than did any other sources of variation.

Comstock[3] found that there was no correlation between weaning weight and postweaning growth in swine when growth rate was calculated at a comparable stage. He suggested that the absence of correlation may be due partly to growth limitations of some of the pigs during the suckling period and partly to different genes controlling growth during the suckling and postsuckling periods. These results are in entire agreement with the observation of Donald (*3*).

Black and Knapp (*1*), in a study of record-of-performance steers, showed that there was no correlation between efficiency of gain before and after weaning. They also noted a low negative correlation in rate of gain before and after weaning. The lack of correlation in both efficiency and rate of gain may have been due to inhibition in growth, during the suckling period, of steers with inherently high efficiency because of limited nutrients, their true inherited efficiency being displayed after weaning when feed was not limited.

MATERIAL AND METHODS

For the present study the records of 58 beef Shorthorn calves from the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., and 180 Hereford calves from the United States Range Livestock Experiment Station, Miles City, Mont., were used. The Shorthorn calves were sired by 8 bulls and the Hereford by 3. The study was made at Beltsville in 1940 from data obtained in 1931–36 for other purposes. The data available from Beltsville included a record of sires, dams, sex, birth weights, weaning weights, and the quantities of milk, grain, and hay consumed by each calf. Milk consumption was calculated by weighing the calf 1 day each week before and after nursing. Both grain and hay were fed individually, and each calf was allowed all it would consume. The grain mixture consisted of 4 parts by weight of cracked corn, 3 parts of crushed oats, 2 parts of wheat bran, and 1 part of linseed meal. The hay fed was No. 2 leafy alfalfa.

In the study involving rate of gain as a basis of selection for breeding, bull and steer calves at Beltsville were used, selection of the bull calf being made at 140 days of age. At that time all bull calves were judged for beef characteristics. Bulls that failed to meet the standard set up were castrated and fed out as steers. Castration was bloodless and had little or no effect on the animals at the time. The dams of the calves also were scored for beef characteristics at irregular intervals over a period of years.

The data available from Miles City included a record of sires, dams, sex, birth weights, weaning weights, and ages at weaning. The calves were weaned at an average age of 180 days and were not grain-fed up to weaning.

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[3] COMSTOCK, R E THE RELATIONSHIP BETWEEN WEANING WEIGHT AND POSTWEANING RATE. [Unpublished]

A correlation study of the Beltsville data was made to determine the effect of consumption of different quantities of milk, grain, and hay on rate of gain during the suckling period. Both the Beltsville and Miles City data were analyzed to determine the variance of daily gain due to sires and sex of calves. Correlations and analyses of variance as outlined by Snedecor (*7*) were used in the analyses of the data.

EXPERIMENTAL RESULTS

In the study of the Beltsville data, the following correlations were found:

	Correlation coefficient[1]
Simple correlation between	
Daily gain and quantity of milk	**0.517
Daily gain and quantity of grain	.209
Daily gain and quantity of hay	.121
Quantities of milk and grain	.142
Quantities of milk and hay	**.388
Quantities of grain and hay	**.456
Multiple correlations between daily gain and quantities of	
Milk and grain	**.591
Milk and hay	**.624
Grain and hay	.211
Milk, grain, and hay	**.641
Partial correlations between daily gain and quantity of—	
Milk (grain and hay constant)	*.619
Grain (milk and hay constant	.188
Hay (milk and grain constant)	*.308

[1] * Statistically significant, *P* 0.05 ** Highly significant, *P* 0.01

The simple correlations indicate that of the feeds consumed the quantity of milk had the greatest effect on rate of gain, followed in order by grain and hay. These last two correlations are not statistically significant, however. There was a slight tendency for the calves that consumed the most milk to consume the least hay and grain. The correlation between the quantities of grain and hay consumed is significant. The multiple correlations indicate that gain per day is associated with these three variables, which account for as much as 41 percent of the variation in rate of gain. Partial correlations show that the quantities of milk and of hay consumed are significantly correlated with daily gain but that the quantity of grain consumed is not significantly correlated with rate of gain. This finding seems to indicate that the more bulky feeds, milk and roughage, have the greatest effect on rate of gain, whereas grain, a more concentrated feed, has little effect. This is in agreement with observations made by animal husbandmen.

TABLE 1.—*Analysis of variance of daily gains, during the suckling period, of 58 Shorthorn calves sired by 8 bulls at Beltsville, Md.*

Variation	Degrees of freedom	Sum of squares	Mean square[1]
Due to sex[2]	2	1.3582	**0.6791
Due to sire effects	14	1.185	.0847
Between calves of the same sex and from the same sire	41	1.8305	.0446
Total	57	4.3744	.0767

[1] ** $P < 0.01$, highly significant

[2] Animals were sorted on the basis of 3 sex differences, there were 14 bulls, 15 steers, and 29 heifers.

Table 1 shows the variance of daily gains, due to sex and sires, of the calves from Beltsville. The analysis shows that differences between sexes are statistically significant, whereas differences between the progeny of different sires are not significant. By analysis of covariance, differences due to sex in the correlations were eliminated. The correlations for calves of the same sex indicate little change in the relationship between quantity of feed and rate of gain. The multiple relationship was reduced from 0.641 to 0.529. The indications are, however, that approximately the same relationship exists between rate of gain and the quantities of milk, grain, and hay consumed among calves of the same sex as among those of the entire population. There were no significant yearly effects owing to the fact that the calves were born during all seasons of the year, causing a larger variation within year than between years.

Further analyses of the Beltsville data show that bull calves consumed significantly more milk than steer calves, but there were no significant differences in the quantities of grain and hay consumed by bulls and steers. This finding seems to indicate that the superior beef characters of the bull calves retained for breeding were due to the greater quantities of milk consumed during the suckling period. As already stated, significant differences in daily gains between the progeny of sires were not demonstrated. Since milk production of the cows bred to different sires may vary either through accidential or premeditated selection of the cows for high milk production, the use of daily gain during the suckling period as a measure of genetic superiority of the progeny for growth is subject to question.

In determining the value of rate of gain as a basis for selection, the analysis of variance of gains, milk consumption, and scores of dams and calves gave the following results: Calves saved for bulls made the most gain up to 140 days of age; they were from cows that gave the most milk up to 140 days; and they were from the poorest scoring cows for beef characteristics. The first two results indicate that selections were associated with nutritional differences and that as a result of such selection the milk production of the herd would be maintained at a high level. This high milk-production level would result in a comparatively low cost per pound of feeder calf. However, the fact that the bull calves selected were from the poorest scoring cows for beef characteristics indicates that the method of selection used would result in poorer beef qualities in the herd. These qualities may not show in the animals selected but would be likely to do so in their progeny. For example, at Beltsville, one of the herd bulls sired thick-fleshed steer calves as shown by the physical analysis of their carcasses. A son of this herd sire, selected as shown above, was out of a cow of poor beef quality but of high milk production. His progeny had significantly more bone and less fat than their sire.

A similar condition of selection was reported by Phillips and Dawson (*6*) in sheep. They found that in the selection of lambs for breeding single lambs were favored over twins, early lambs over late lambs, and heavy lambs at birth over light lambs, although no conscious effort was made to favor any of these groups.

As already stated, an analysis-of-variance study was made of the daily gains of 180 calves sired by 3 bulls at Miles City, Mont. Black and Knapp (*2*) observed significant differences, in rate of gain subsequent to weaning, in the progeny of the three bulls. Table 2 shows the analysis of variance of the daily gains prior to weaning. With these calves, as well as those from Beltsville, significant differences were observed between sexes but not between the progeny of different sires. The small differences that were observed between the progeny of different sires are probably the result of the nutritional level rather than of genetic variation.

TABLE 2 *Analysis of variance of daily gains, during the suckling period, of 180 Hereford calves sired by 3 bulls at Miles City, Mont.*

Variation	Degrees of freedom	Sum of squares	Mean square [1]
Due to sex	1	0.4080	**0.4080
Due to sire effects	4	2788	.0697
Between calves of the same sex and from the same sire	174	6.6785	0384
Total	179	7.3653	0411

[1] **P 0.01 highly significant

Since the study showed no significant difference in rate of gain during the suckling period in the progeny of different sires, it may be concluded that rate of gain during this period is no indication of the genetic superiority of the calves. However, determination of rate of gains during the suckling period would be of value if this gain were shown to have a close correlation with gain after weaning. A correlation study on record-of-performance Hereford steers at Miles City showed a coefficient of 0.023 (nonsignificant) between rate of gain before and after weaning. This observation is in agreement with that of Black and Knapp (*1*) with cattle, and with those of Donald (*3*) and Comstock [4] with swine. The conclusion seems sound, therefore, that there is little or no relationship between rate of gain during the suckling period and rate of gain after weaning. Consequently, as a method of genotypic selection, observations on rate of gain prior to weaning have little value.

SUMMARY

The study reported in this paper was made to determine the factors influencing rate of gain in beef calves during the suckling period and to ascertain the value of rate of gain during this period as a basis of selection for breeding.

A statistical study of the daily gains during the suckling period was made on 58 beef Shorthorn calves at the United States Department of Agriculture Beltsville Research Center, Beltsville, Md.; and 180 Hereford calves at the Range Livestock Experiment Station, Miles City, Mont. The work was carried on at Beltsville in 1940 with data obtained in 1931-36 for other purposes. The influence, on the daily gains, of sires, dams, sex birth and weaning weights, ages at weaning, and milk, grain, and hay consumption, was determined.

Partial correlation studies indicated that under the conditions of this study, milk consumption had the greatest influence on rate of gain, followed in order by hay and grain. The combined influence of these three variables accounted for 41 percent of the variation in rate of gain during the suckling period.

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[4] See footnote 3

The analysis-of-variance study indicated that differences between the progeny of different sires cannot be demonstrated by rate of gain during the period prior to weaning. Furthermore, evidence is presented which shows that there is little or no relationship in rate of gain before and after weaning. Sex was found to have a significant influence on rate of gain. The study indicated that when selection of breeding animals was made during the suckling period, the calves selected were those that made the greatest gain and that they were from cows which gave the most milk but scored the poorest for beef characteristics.

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HISTOCHEMICAL DETECTION OF GLYCOGEN IN BLOOD CELLS OF THE SOUTHERN ARMYWORM (PRODENIA ERIDANIA) AND IN OTHER TISSUES, ESPECIALLY MIDGUT EPITHELIUM[1]

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INTRODUCTION

Although the storage of carbohydrate in the form of glycogen is apparently related to the metamorphic processes of holometabolous insects, little is known regarding the processes of intermediary carbohydrate metabolism that occur in insects. Virtually nothing is known as to whether a given status of intermediary carbohydrate metabolism may influence the degree of susceptibility or resistance of an insect to a poison or to an insecticidal procedure. It has been reported (*75*, *91*)[2] that the inclusion of brown sugar or molasses in certain insecticidal sprays or baits may result in an increased kill of certain insects. The added sugar or molasses is believed to cause the poison to be held on the sprayed foliage for a longer time, thereby increasing the amount of poison that the insect ingests. Richardson (*91*) has reported that the addition of brown sugar or molasses increases the solubility of an arsenical insecticide in the spray. In such a case an increase in the carbohydrate content of the gut might cause more poison to be held in solution in the digestive juices of the insect's alimentary tract, whether the carbohydrates were ingested along with or prior to the poison. If this should occur, an increase in the carbohydrate content of the insect's blood or hemolymph (i. e., an alimentary hyperglycemia) might produce a greater solubility of an arsenical poison in the blood, and hence possibly a greater effectiveness of the poison upon the insect's tissues. Ingestion and absorption of sufficient carbohydrate might itself have an injurious or lethal effect upon at least certain species of insects (*19*). Thus, from the standpoint of toxicology as well as physiology, further knowledge of the occurrence, distribution, and metabolism of carbohydrates in insects is required.

In a study of the blood cells of the southern armyworm (*Prodenia eridania* (Cram.)) glycogen was detected in certain of the blood cells and in some of the large midgut epithelial cells that frequently occurred in blood smears from first and second instars. This paper is the report of a qualitative investigation of the occurrence of histoproofs to the Bauer method. Negative counterproof results indicated that the glycogen had been digested by the ptyalin of the saliva, and that the positive Bauer reaction was not caused by mucin.

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[1] Received for publication February 21, 1941.

[2] Italic numbers in parentheses refer to Literature Cited, p. 288. No reference is made to publications appearing after 1939, the year the manuscript was written.

IDENTIFICATION OF INSTARS

To identify the different instars used in this investigation, head-capsule measurements were utilized. A larva was considered to be of a given instar when its head-capsule measurement (width) approximated the mean value of head-capsule measurements for that instar as determined by Mayer and Babers,[3] whose data show that the southern armyworms reared at this laboratory develop through six instars. Generally, the mature larva stops eating a short time before entering the ground. The age within a given instar was determined, in some cases, by isolating a larva of known instar, noting when it molted, and utilizing it at a determined subsequent hour. The age of a first instar was determined from the time of hatching. Similarly, ages of pupae and adults were determined, respectively, from the times of pupation and emergence.

STARVATION AND FEEDING

In starvation and feeding experiments upon newly hatched larvae, insects that hatched at the same time were separated into six groups. One group was starved. Each of the other groups was fed one of the following diets: (1) Turnip leaves, (2) gelatin gel, (3) gelatin gel containing glucose, (4) cornstarch paste, and (5) cornstarch paste containing glucose. A mold soon developed on the gelatin, with or without glucose, which was fed to the larvae in the form of a gelled layer covering the insides of the beakers holding the larvae. The cornstarch diets were supplied as a thick paste prepared by boiling.

Sixth instars were also starved or fed individually the following diets: (1) Turnip leaves, (2) collard leaves, (3) white cabbage leaves, (4) green soybean leaves, (5) yellowed soybean leaves, (6) cornstarch paste containing glucose, and (7) cornstarch-glucose paste in turnip-leaf sandwiches. In these experiments the foods were supplied to the larvae qualitatively, but in general the ratio of glucose to cornstarch was about 1 to 10 In other experiments larvae were fed larger amounts of glucose (up to about 1 part to 2 parts of boiled cornstarch). After having access to cornstarch-glucose-turnip leaf sandwiches for a given length of time (usually about 16.5 hours), and an interval of 2 to 3 hours' starvation, some of these larvae were ligatured.[4] Ligatured and unligatured larvae were then fed cornstarch-turnip leaf sandwiches.

The periods of starvation and feeding of the young and the mature larvae differed. As a rule the newly hatched larvae were starved until dead or were fed for about 3 days. The mature larvae that were to receive the special diets were kept without food for about 30 hours and were then fed for about 4 days. The starved mature larvae were kept without food up to about 70 hours. At the end of the starvation and feeding periods, individual larvae were used for glycogen detection.

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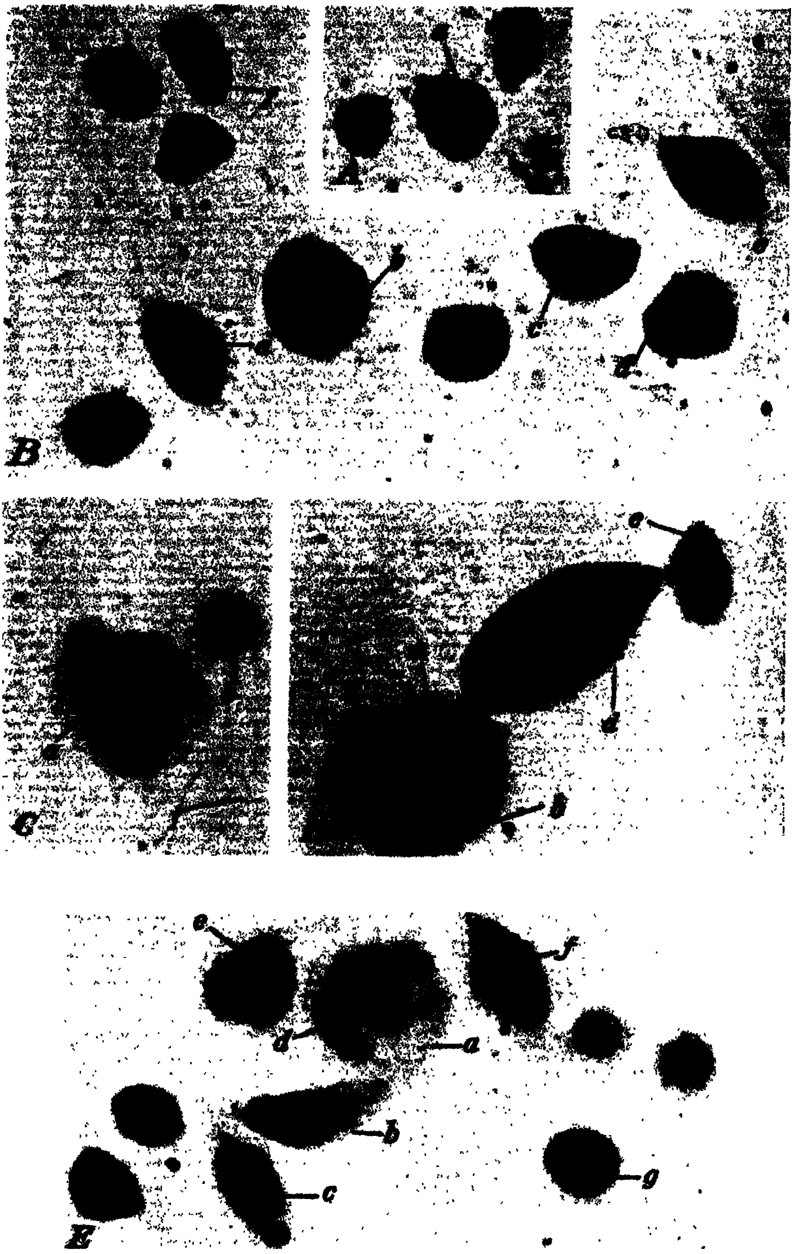
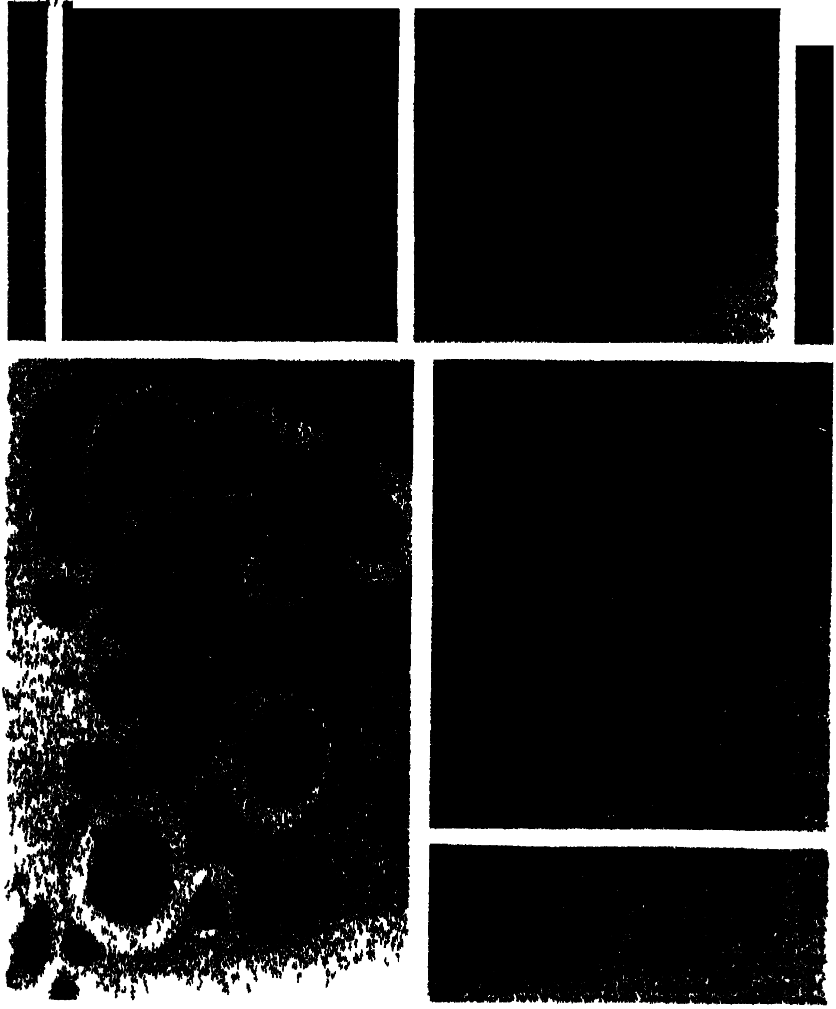
[3] MAYER, E L, and BABERS, F. H HEAD CAPSULE MEASUREMENTS OF SOUTHERN ARMYWORM LARVAE (PRODENIA ERIDANIA) [To be published]

[4] These larvae served as controls in experiments on the effects of administered poisons on blood-cell glycogen



Blood cells from larvae of *Prodenia eridania* that had been fed turnip leaves: *A*, Second instar; *B*, fourth instar; *C*, *D*, and *E*, fifth instars. Glycogen inclusions are shown in *A* at *a*; in *B* at *a–f*; in *C* in a plasmatocyte, *a*, but not in the small cell *b*, which was more deeply stained with thionin; in *D* at *a* and *b* in the plasmatocyte *c*, but not in the cystocyte *d* or in the cell *e*; in *E* conspicuously in the plasmatocytes *a*, *b*, and *c*, as at *d*, less conspicuously in the plasmatocytes at *e* and *f*, but not in the other cells. In *E* note the polar locations of the glycogen inclusions in the fusiform plasmatocytes *b* and *c*; a metaphase is shown at *g*. Blood smears, Bauer technique; oil immersion, 10× ocular, 93× objective.



Blood cells from larvae of *Prodenia eridania*: *A* and *B*, Sixth instars fed turnip, collard, and cabbage leaves; *C*, a greatly tapered prepupa reared on turnip leaves; *D*, a day-old pupa reared on turnip leaves. In *A* glycogen inclusions are shown at *a* and *b* in plasmatocyte *c* and at *d* in plasmatocyte *e*, but not in plasmatocytes *f* and *g* or in cell *i*, a type that has not been observed to contain glycogen; a nuclear band, *h*, is visible in *g*. In *B* glycogen inclusions occur in plasmatocytes *a*, *b*, and *c*, but not in plasmatocytes *d* and *e* or in cell *f*, which is the same type as cell *i* in *A*. In *C* granular glycogen inclusions are shown in the cystocytes *a*, *b*, and *c*, and larger inclusions in plasmatocytes *d*–*m*, particularly in *k*, *l*, and *m*; glycogen also appears in some of the small cells, as at *n* and *o*, the nucleus of a plasmatocyte appears banded at *p*. In *D* glycogen inclusions are shown at *a*–*e*. Blood smears, Bauer technique; oil immersion, 10X ocular, 98X objective.

THE GLYCOGEN COUNT AND THE GLYCOGEN INDEX

A glycogen count was obtained from a blood smear by taking a certain number of cells at random and classifying each cell according to whether it contained one, two, three, or four or more glycogen inclusions. All the cells observed in each of a series of microscopic fields, visible with high dry magnification (about 660×), were counted until a total of 400 cells had been classified. From these figures the percentages of the blood-cell population of the smear that contained the various numbers of glycogen inclusions and the total percentage of cells that contained glycogen were calculated. The percentages of cells containing the various numbers of glycogen inclusions constitute what is here called the glycogen count. The total percentage of cells that contain glycogen constitutes the glycogen index.

RESULTS

BLOOD-CELL GLYCOGEN IN INSECTS FED TURNIP LEAVES

Glycogen was not detected in the blood cells of newly hatched larvae by any of the methods used. In blood cells of larvae fed turnip leaves glycogen was first observed 18.5 hours after hatching and then in only one cell in only one larva. In one other larva glycogen was detected in several blood cells at some time between 23 and 39.5 hours after hatching. Blood-cell glycogen was usually not observed in older first instars or in second instars. When glycogen did occur, it was seen in very few blood cells of the first instars and in more cells of the second instars.

Plate 1, *A*, shows a glycogen-containing blood cell from a second instar. The youngest larva to show glycogen in a considerable number of blood cells was a second instar just after molting. In general, later instars that possessed blood-cell glycogen tended to show it in increasing numbers of cells with each succeeding instar (fig. 3). Plates 1, *B*-*D*, and 2, *A* and *B*, show some glycogen-containing blood cells from fourth, fifth, and sixth instars. Relatively large numbers of blood cells contained glycogen in sixth instars, prepupae, and the very young pupae that did exhibit blood-cell glycogen. In general, blood-cell glycogen tended toward a maximum at the prepupal stage of development, as shown by the variation of glycogen index during larval development and metamorphosis (fig. 3). During metamorphosis the blood-cell glycogen decreased until in later pupal life it reached a minimum, and sometimes seemed to disappear entirely. Plate 2, *C* and *D*, shows glycogen-containing blood cells from a greatly tapered prepupa and a day-old pupa. The decrease of pupal blood-cell glycogen was associated with a marked decrease in the number of cells that appeared in the blood smears. In the smears from old pupae it was frequently difficult to find any blood cells; the few that were found usually contained little or no glycogen. In the imago blood-cell glycogen was rarely observed.

Individual differences in the occurrence of blood-cell glycogen were large in all the instars, particularly in the earlier instars and among individuals from different batches of larvae. No blood-cell glycogen could be detected in some larvae.

GLYCOGEN IN MIDGUT EPITHELIAL CELLS OF LARVAE FED TURNIP LEAVES

No glycogen was observed in the midgut epithelial cells of newly hatched larvae. Midgut-cell glycogen was observed earliest in a single turnip-leaf-fed larva 6 hours after hatching. It was also observed in several first instars 11, 24, 26, 28.5, 39, and 47 hours old, but not in other larvae of these ages. Plate 3, *A* and *B*, shows glycogen in midgut epithelial cells from a first instar. In this instar the glycogen in these cells generally appeared earlier after hatching and more frequently than did glycogen in the blood cells.

Glycogen also occurred in the midgut epithelial cells of the later instars, although individual differences, as indicated by the smeared gut cells, were quite marked. Plate 4, *A–C*, shows glycogen in some midgut epithelial cells from a third and a sixth instar. As in the occurrence of blood-cell glycogen, some individual insects appeared to have considerable midgut-cell glycogen, whereas others possessed none. In none of the larvae did glycogen appear in all the smeared epithelial cells.

EFFECTS OF STARVING LARVAE AND OF FEEDING THEM DIFFERENT FOODS

Newly Hatched Larvae

When newly hatched larvae were starved, no glycogen was observed in either their blood cells or their midgut epithelial cells, even up to the time of death, which was usually not more than 48 hours after hatching. In plate 3, *C*, are shown midgut epithelial cells from a starved first instar.

As has been stated, midgut-cell glycogen was observed in some of the first instars fed turnip leaves but not in others, and blood-cell glycogen was observed in only one or two of these larvae and then only in one or two cells in a smear.

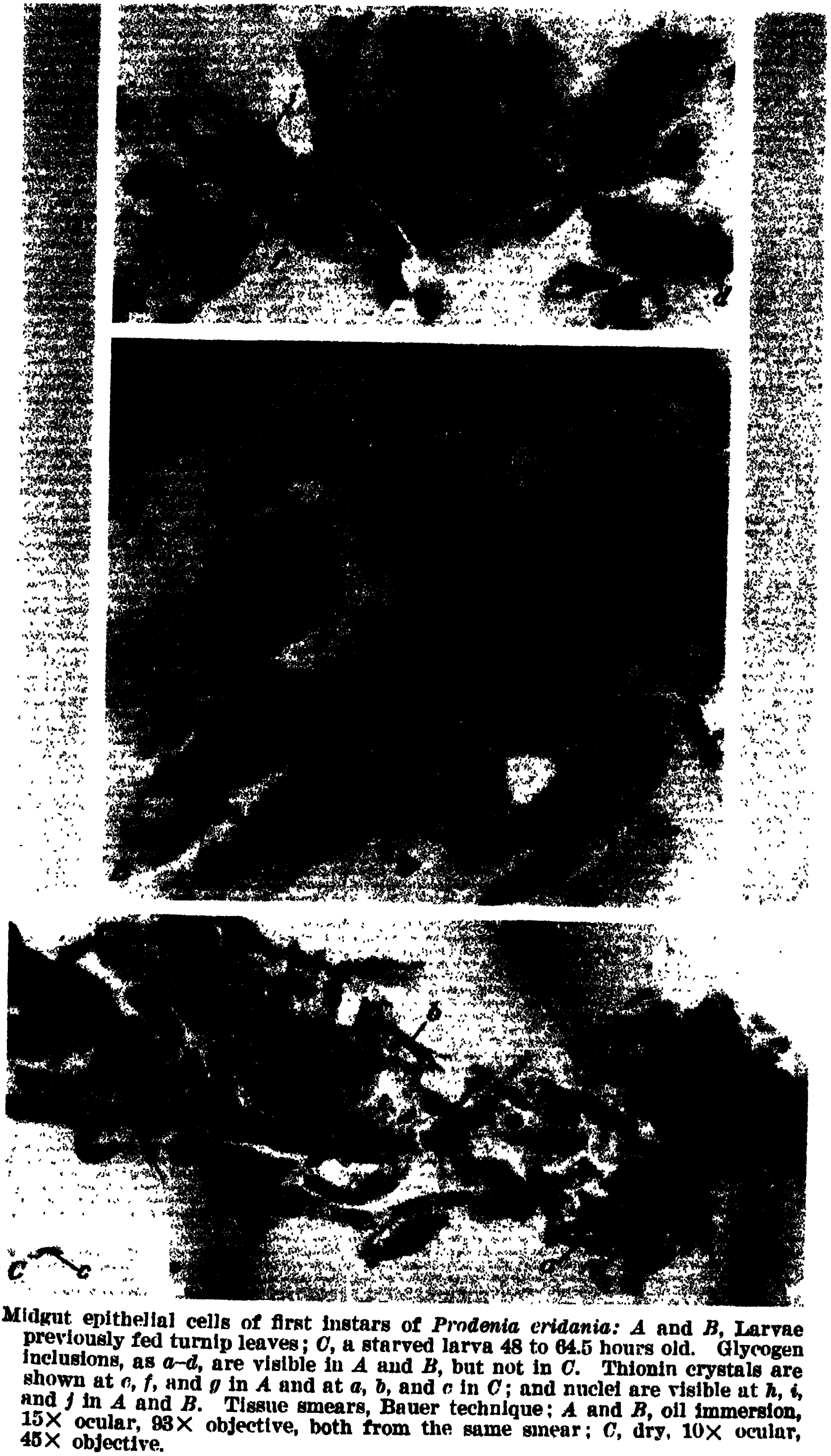
Gut-cell glycogen was observed in some larvae fed cornstarch or cornstarch plus glucose, but no blood-cell glycogen was observed in any of these larvae. No gut-cell or blood-cell glycogen was observed in any of the larvae fed gelatin (plus the mold that developed on the gelatin in the course of the experiment). Midgut-cell glycogen was observed in some of the larvae fed gelatin-glucose (plus the developed mold), but blood-cell glycogen was not detected.

Sixth Instars

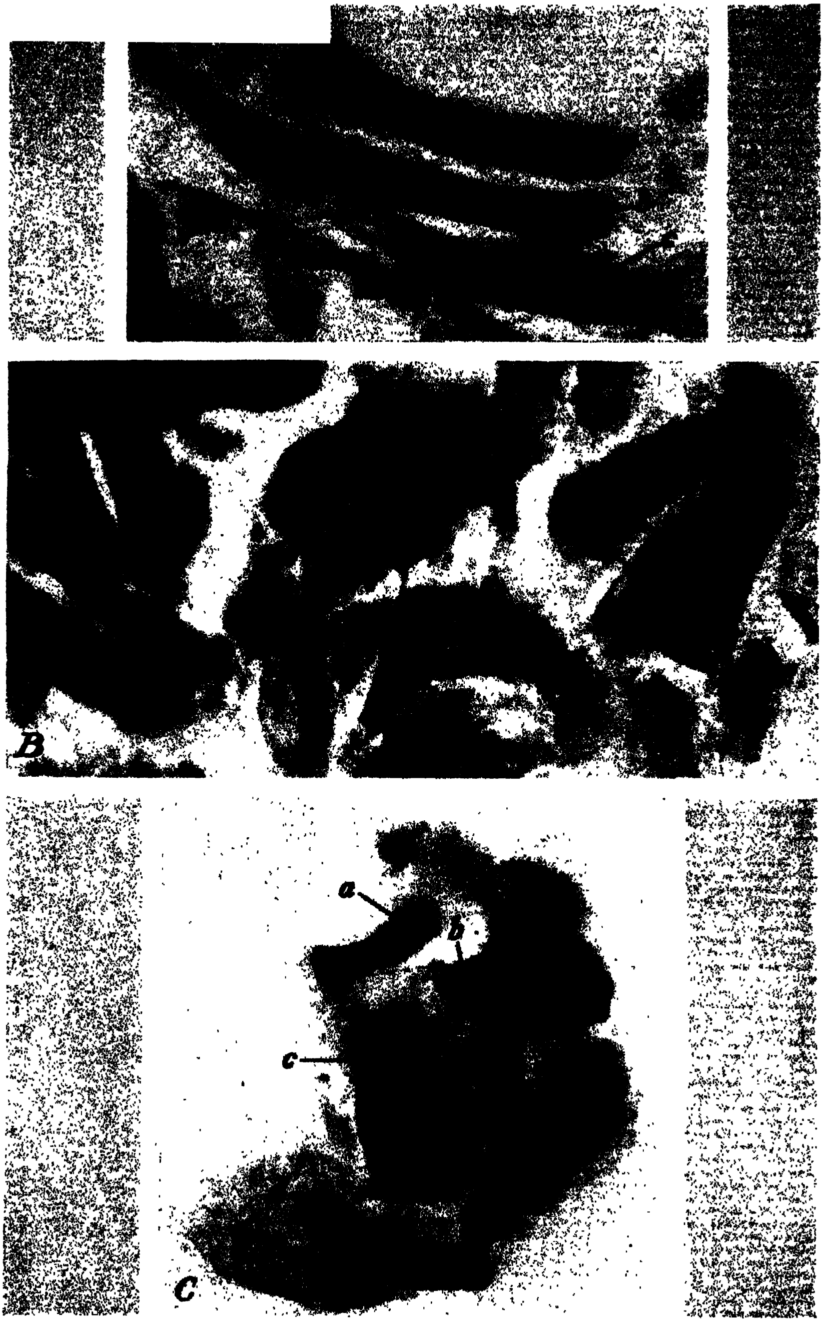
When sixth instars were taken from diets of turnip and collard leaves and starved for different periods up to 70 hours, glycogen was observed in fewer blood cells the longer the period of starvation until, at about 24 to 40 hours, depending upon the individual insect, it was no longer detected.

Sixth instars reared on turnip leaves appeared to have more blood-cell glycogen than those reared on collard leaves or, especially, on white cabbage leaves. In larvae fed soybean leaves blood-cell glycogen appeared to be variable, but in general it seemed to be greater when green than when yellowed leaves were fed. Blood-cell glycogen increased when cornstarch-glucose paste, alone or in turnip-leaf sandwiches, was fed to larvae reared on turnip, collard, or white cabbage leaves. Larvae that were fed the smaller quantities of glu-

PLATE 3



Midgut epithelial cells of first instars of *Prodenia eridania: A* and *B*, Larvae previously fed turnip leaves; *C*, a starved larva 48 to 64.5 hours old. Glycogen inclusions, as *a–d*, are visible in *A* and *B*, but not in *C*. Thionin crystals are shown at *e*, *f*, and *g* in *A* and at *a*, *b*, and *c* in *C*; and nuclei are visible at *h*, *i*, and *j* in *A* and *B*. Tissue smears, Bauer technique; *A* and *B*, oil immersion, 15× ocular, 93× objective, both from the same smear; *C*, dry, 10× ocular, 45× objective.



Midgut epithelial cells of third and sixth instars of *Prodenia eridania: A* and *B*, Third instars reared on turnip leaves; *C*, sixth instars reared on turnip, collard, and cabbage leaves. Glycogen inclusions are shown in cells *a*, *b*, and *c* in *A*; in many of the cells in *B*; and in *C*, those at *a–d* having polar locations. Nuclei are scarcely visible. Tissue smears, Bauer technique; dry, 10× ocular, 45× objective. *C* is from the same larva that yielded the blood smear shown in plate 2 *A*.

Blood cells from sixth instars of *Prodenia eridania* that had been fed turnip-leaf cornstarch sandwiches containing glucose. *A* and *B* from a larva that had eaten a sandwich containing less glucose than in the sandwich eaten by the larva represented in *C* and *D*. In *A* and *B* glycogen appears in all the cells shown though it is seen more clearly in some, as at *a*, than in others, as at *b* and *c*; the inclusions of the fusiform plasmatocyte *B*, *d*, have approximate polar locations. *C* shows especially well the inclusions in the cystocytes *a*, *b*, and *c*; those of the other cells, as at *d–g*, appear as black areas but the cell bodies, as at *h*, *i*, appear very faint; an oenocytoid appears at *l*. *D* shows glycogen inclusions as at *a–e* and an oenocytoid at *f*; the cell bodies are more distinct than in *C*; most of the cells contain glycogen inclusions but not the oenocytoid *f*. Blood smears, Bauer technique. *A* and *B*, oil immersion, 10X ocular, 93X objective; *C* and *D*, dry, 10X ocular, 45X objective.

Midgut epithelial cells from a sixth instar of *Prodenia eridania* fed turnip leaf-cornstarch sandwich containing much glucose. Glycogen inclusions are shown in most of the cells, as in the parts lettered *a–k*; the inclusions at *c* and *f* are intracellular, although the enclosing cytoplasm is hardly visible in the photomicrograph. Tissue smear, Bauer technique, dry, 10X ocular, 45X objective.

cose (pl. 5, *A* and *B*) usually possessed glycogen in fewer blood cells than did the larvae receiving the larger quantities (*C* and *D*). In one of the larvae that ingested the larger amounts of glucose approximately 85 percent of the blood cells contained glycogen. Some of the cells from these larvae contained numerous and often relatively large glycogen inclusions. In general, it appeared that the greater the amount of glucose in the food and the longer the time of ingestion, the greater was the amount of blood-cell glycogen.

The few observations made also indicated that the feeding of large amounts of glucose in cornstarch-turnip leaf sandwiches to sixth instars increased their midgut-epithelial-cell glycogen (pl. 6).

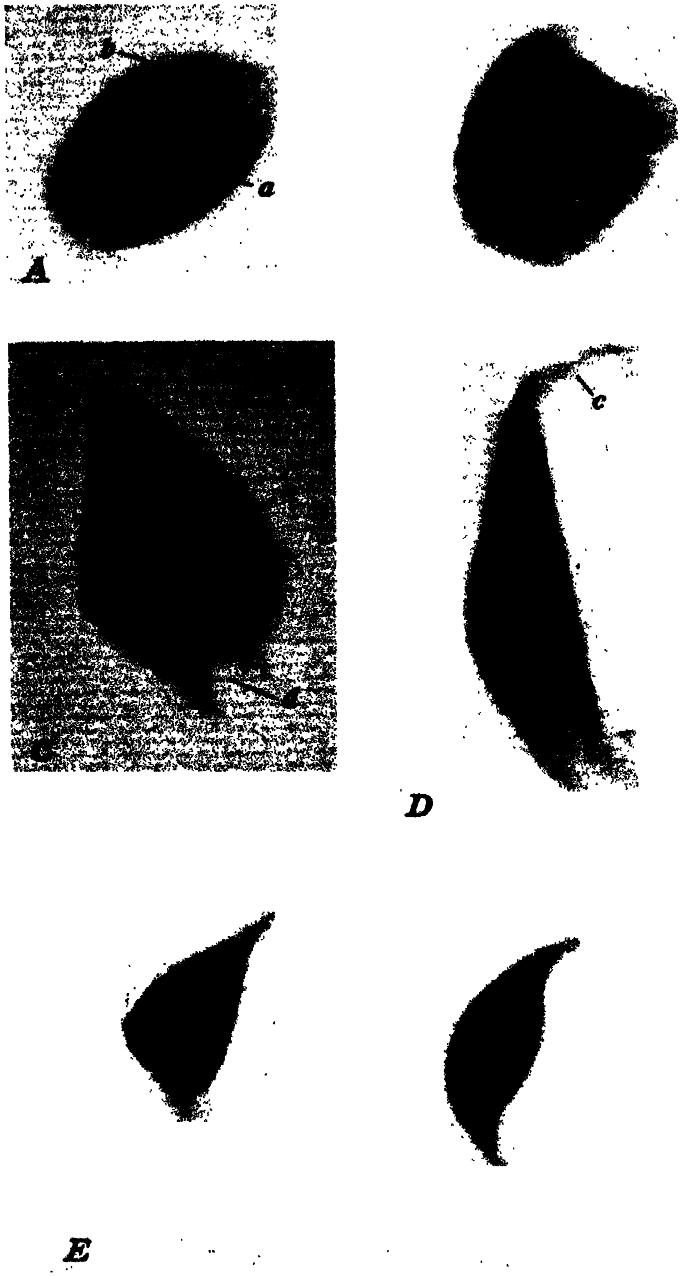
KINDS OF BLOOD AND MIDGUT EPITHELIAL CELLS CONTAINING GLYCOGEN

In none of the blood smears, whether stained by the Bauer, the Best, or the Lugol method, were all the different types of blood cells identified with certainty. No attempt will be made in this paper to present a complete classification of the blood cells of the southern armyworm, since this subject is being investigated as a separate study. The blood-cell types mentioned here are described and named in accordance with a tentative classification.

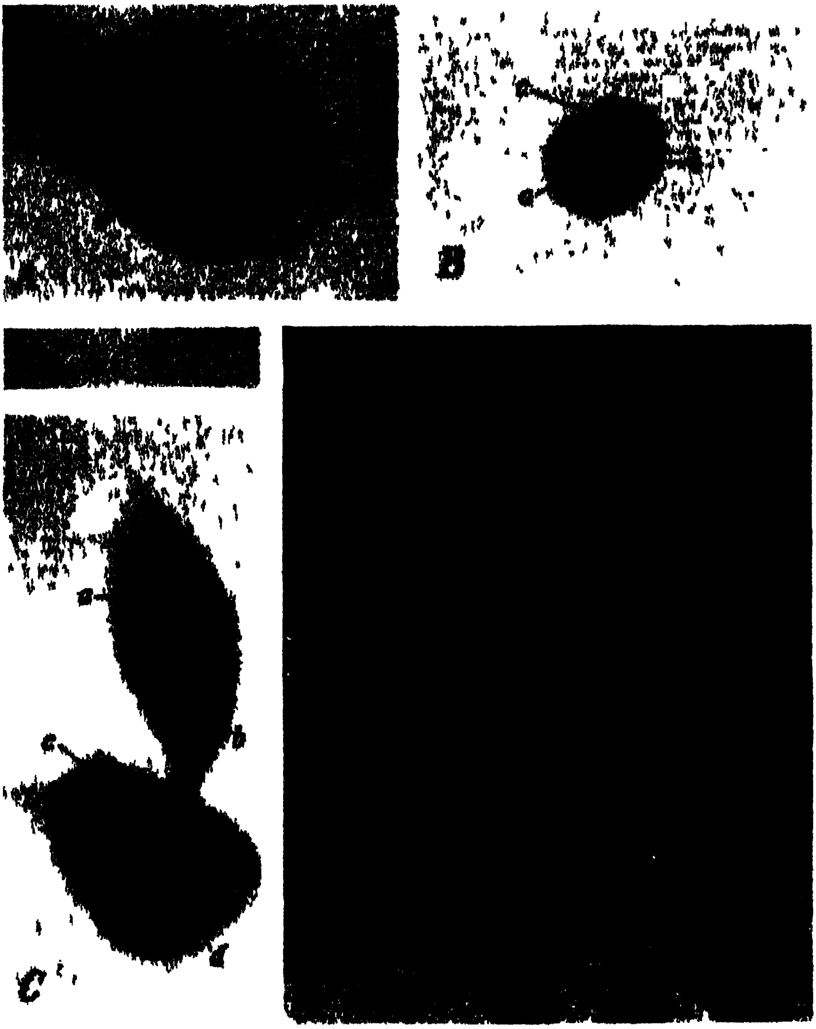
When larvae were reared on turnip or collard leaves, only a part of their smeared blood cells were observed to contain glycogen. In the glycogen-stained smears from first, second, or third instars the identification of blood-cell types was particularly difficult, and in some was impossible. In smears from the later instars, however, especially the fifth and sixth, and from the prepupal and early pupal stages, the blood-cell types, here called plasmatocytes and cystocytes, have been definitely identified among the glycogen-containing cells. These cell types will be described here briefly, in terms of their appearance when obtained from insects fixed in water at 60° C. for 5 minutes and stained with Wright's blood stain.

The plasmatocytes are flattened blood cells (pl. 7, *D*) with outlines that may be markedly fusiform (pls. 7, *D* and *E*; 8, *D*), fusiformoid (pl. 7, *A*)—i. e., tending to be fusiform but without definitely pointed spindle ends—ovoid, round, or, at times, polymorphic (pl. 7, *B*). The nucleus is eosinophilic and punctate (pl. 8, *B*), has well-defined chromatin granules, and frequently has suggestions of chromatin threads. It usually contains several bodies that are considered to be nucleoli. The nuclear structure may be obscured by overlying cytoplasm (pl. 7, *A* and *B*), but there is much variation in the degree to which this may occur. The nuclear outline varies from nearly round (pls. 7, *E*; 8, *A*–*C*) to an elongated oval (pl. 8, *D*), but most frequently it is moderately ovoid. Often the nucleus has a grooved or notched appearance (pl. 8, *C*). A band of basophilic cytoplasm frequently stretches across the visible surface of the nucleus (pl. 8, *A*, *B*, and *D*), and in some cases has been interpreted to be basophilic cytoplasm in the nuclear groove. The cytoplasm contains a poorly developed to very well developed vacuolization (pl. 7, *A*). When poorly developed, the cytoplasmic vacuolization tends to consist of a few irregular, colorless vacuolar regions of different sizes, which may appear to be disconnected or irregularly channeled, or both. The poorly developed vacuolization has been observed to predominate in the plasmatocytes of the earlier instars. When the vacuolization is very well developed, the cytoplasm appears to be filled with many small, uniformly distributed, colorless vacuoles, not very distinct individually. Well-developed vacuolization has been observed chiefly in cells from the late instars, particularly the sixth. Most of the observed plasmatocytes of the older larvae, however, exhibited cytoplasmic vacuolizations intermediate between these two extremes (pls. 7, *B* and *E*). The amount of cytoplasm varies from cell to cell. In general, plasmatocytes with smaller amounts of cytoplasm relative to nuclear size tend to be the smaller cells and to possess the more poorly developed vacuolization, whereas plasmatocytes with larger amounts of cytoplasm relative to the nucleus tend to be the larger cells and to possess the better developed vacuolization; exceptions to this rule also occur. Frequently the cytoplasm of the plasmatocyte also contains one or more gross colorless inclusions (pl. 7, *C*), which lie in the finely vacuolized cytoplasm and often are located toward the spindle ends of the fusiform cell. Many plasmatocytes from normally fed larvae do not exhibit these large vacuoles, but those that do may contain one or perhaps two large vacuoles, and occasionally three or more. The plasmatocytes lie on their flat sides in blood smears (pl. 7, *D*). Their nuclei are flattened bodies, and it is probable that the larger of the cytoplasmic vacuoles are also somewhat flattened. The cell peripheries of the plasmatocytes vary in distinctness and often tend to be very indistinct (pls. 7 and 8). The plasmatocyte has not been observed to possess a definitely visible cellular membrane. The surface layer of the cytoplasm seems to be a sort of labile ectoplasm, different from the vacuolized endoplasm, especially in cells that have begun to undergo or have undergone a change in form. Observations upon blood from mature larvae, untreated or incompletely treated by heat, showed that the plasmatocytes readily undergo passive-active transformations (*115*). The plasmatocytes have been observed in the various instars, the prepupa, the pupa, and the adult of the southern armyworm. As indicated by blood smears, they appeared to attain an optimum of cytoplasmic development in the mature larva or prepupa and to decrease in numbers in the pupa.

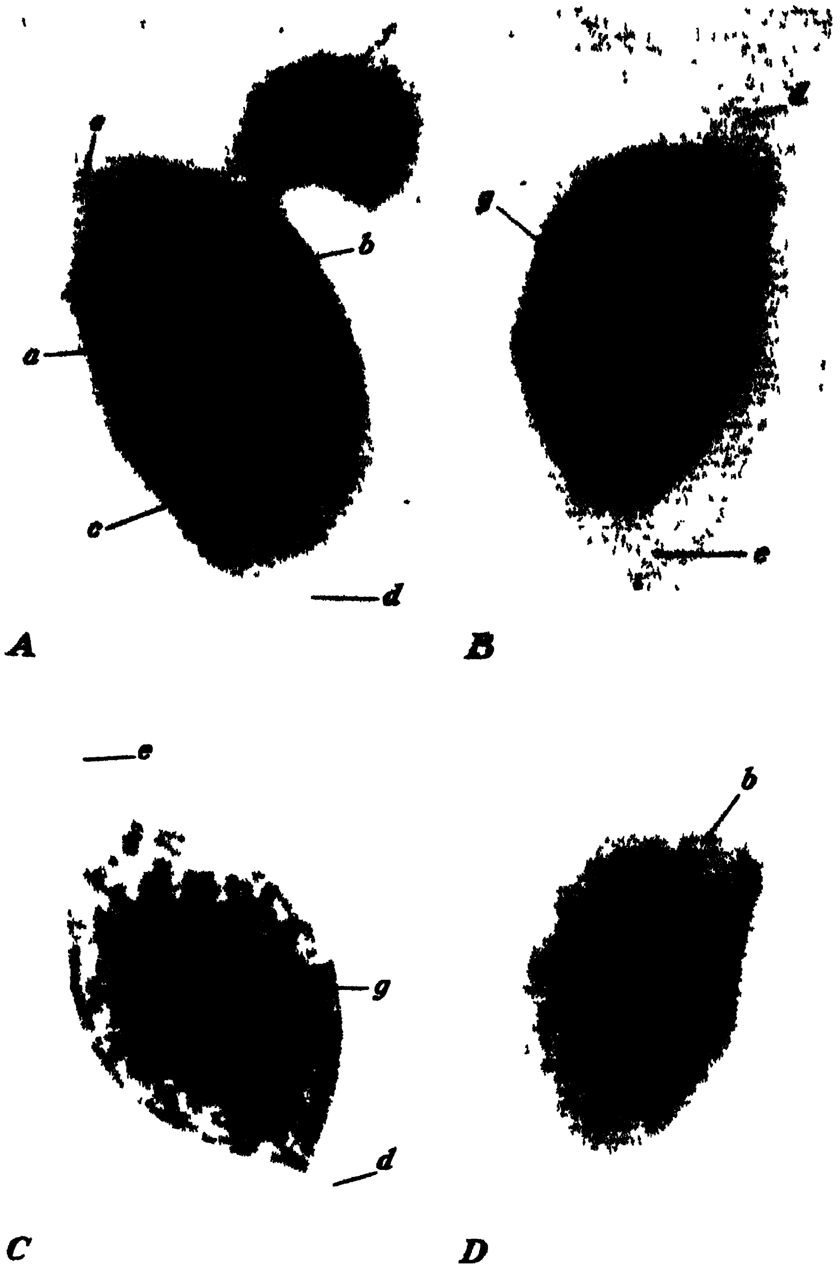
The cystocytes (pl. 9) are also slightly flattened but are much thicker than the plasmatocytes. Their outlines may also be fusiform, fusiformoid, ovoid, round, or polymorphic. The nucleus is usually eosinophilic but sometimes appears to be basophilic; it is more or less punctate, is usually ovoid to round, and is frequently much obscured or completely hidden by the cytoplasmic inclusions. The cytoplasm is basophilic, but contains large eosinophilic, saclike inclusions, to which the term "cyst" is here applied (pl. 9, *b* and *c*). At times the perinuclear cytoplasm seems to cloak the nucleus with a spurious basophilia. The main body of the fully developed fusiform cystocyte may become bloated with eosinophilic cysts, whereas the spindle terminations of the cell remain thin, lightly basophilic, and completely free of cysts. Sometimes, however, it is difficult to detect the spindle ends of these cells. The number of cysts and the degree of eosinophilia in a cell vary greatly. The cystocytes do not exhibit distinct cellular membranes, but, like the plasmatocytes, appear to possess a sort of labile ectoplasm, which can be more distinctly observed in cells undergoing or having undergone form changes.



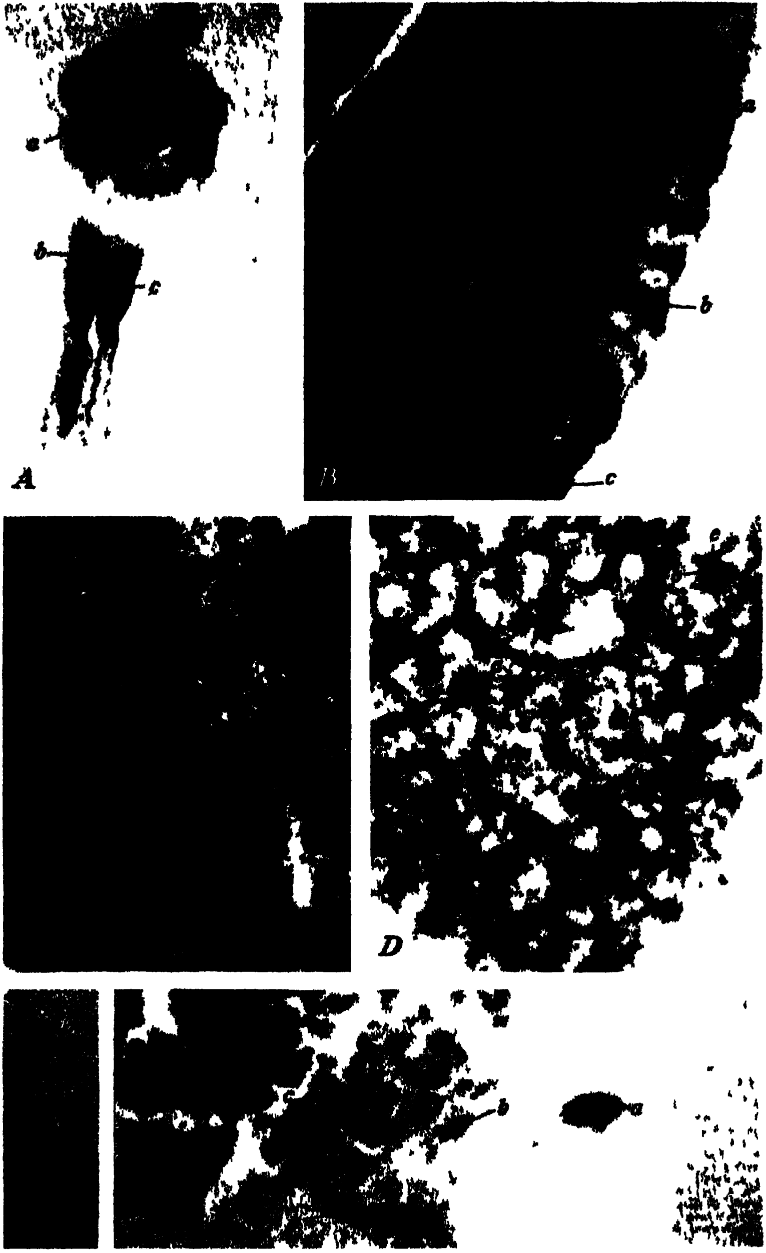
Plasmatocytes, showing finely vacuolized cytoplasm: *A*, Fusiformoid plasmatocyte; *B*, polymorphic plasmatocyte; *C*, *D*, *E*, fusiform plasmatocytes. In each case the vacuolized cytoplasm, as *b* in *A*, tends to obscure the nucleus, as *a* in *A* and *C*, and there is no distinct cell membrane. The plasmatocyte in *C* contains a large cytoplasmic vacuole, *d*, which resembles a glycogen inclusion in form and location; the other plasmatocytes are without gross vacuoles. In *D* the vacuolization is visible only at the ends of the cell; one of the spindle ends, *c*, happens to be folded in such a way as to demonstrate the flattened form of the cell. Blood smears, Wright's technique; oil immersion, 15× ocular, 93× objective.



Plasmatocytes showing banded and notched appearance of nuclei. *A* and *B* represent the same cell. *A* shows the deeply stained nucleus, *a*, across which extends a cytoplasmic band, *b*, and the relatively large area of thinly spread vacuolized cytoplasm *c*, no distinct cell membrane appears. *B* shows the punctate and banded structure of the nucleus more clearly, *a* is a chromatin mass, *b* is the cytoplasmic band, and *c* is the nuclear notch in which the cytoplasmic band appears to be. *C*, Two plasmatocytes, in one, *a*, the nucleus presents a notch, *b*, which is associated with a band not visible in the illustration in the other, *c*, is a large cytoplasmic vacuole, *d*, in a finely vacuolized cytoplasm, the cells possess no distinct cell membranes. *D*, Plasmatocyte, *a*, containing a nucleus across which stretches a cytoplasmic band, *b;* the extremely elongated, snakelike cell, *c*, here called a nematocyte, also has a banded nucleus *d;* glycogen inclusions appear as dark masses in the cytoplasm of a number of cells, as at *e–j;* those in cells *a*, *k*, and *l* occupy polar positions, the ends *m* and *n*, of the nematocyte, *c*, extend for some distance out of the picture. Blood smears, *A*, *B*, and *C*, Wright's technique, 15X ocular, *D*, Bauer technique, 10X ocular, oil immersion, 93X objective.



Cystocytes showing main body of the cytoplasm loaded with cysts as *b* and *c*. In *A* the spindle ends, *d* and *e*, of the cystocyte, *a*, contain no cysts, and are very faintly vacuolized and very thin; a plasmatocyte occurs at *f*. In *B* the spindle ends, *d* and *e*, are finely and faintly vacuolized, and the deeply stained punctate nucleus, *g*, is partly obscured by the surrounding cystic cytoplasm. No distinct cell membrane appears in *A* or *B*. In *C* the spindle ends, *d* and *e*, are almost invisible, and the punctate character of the deeply stained nucleus, *g*, is not visible. The cystocyte in *D* illustrates the cystic character of the main body of the cytoplasm, a cyst being especially evident at *b*, the spindle ends of this cell are invisible. The dark lines between the cysts represent basophilic cytoplasm. Blood smears, Wright's technique; oil immersion; 15X ocular, 93X objective.



Various tissues from smears of a sixth-instar *Prodenia eridania* fed turnip-leaf-cornstarch-glucose sandwich: *A*, Ganglion of the ventral nerve cord, large black areas at *a*, *b*, and *c* indicate a positive reaction for polysaccharide. *B*, Labial gland; *a–e* are polysaccharide inclusions, and the dark area, *f*, indicates a positive Bauer reaction. *C*, Region of a Malpighian tube, the edge of which appears at *a*; tissue appears to be loaded with polysaccharide, as at *b*, and to contain heavier polysaccharide inclusions, as at *c*, *d*, and *e* *D*, Part of fat body, showing polysaccharide distributed throughout; the larger inclusions, as at *a*, *b*, and *c*, are in areas of thionin-stained protoplasm, as *d*, *e*, and *f*. *E*, Gonad, showing polysaccharide inclusions, as at *a–d*. Bauer technique, counterstained with thionin; dry: *A*, 10× ocular, 10× objective; *B*, *C*, *D*, and *E*, 10× ocular, 45× objective.

Like the plasmatocyte, the cystocyte is capable of undergoing passive-active transformations. Cystocytes have not been observed in the early instars. They appear later and tend toward a maximum of cytoplasmic development and of numbers at about the prepupal or pupal stage. They decrease to a minimum or completely disappear in the pupal period.

The plasmatocytes and the cystocytes were the chief blood-cell types in which glycogen was observed when larvae were not fed glucose but were reared on turnip leaves or fed turnip or collard leaves for a considerable time. When the turnip or collard leaves were supplemented by a moderate carbohydrate diet (for example, cornstarch or cornstarch with a small amount of glucose) for only a short time, glycogen was also observed occasionally in some of the other types of blood cells. But when larvae were fed the larger amounts of glucose for a sufficiently long time, glycogen could be observed in most of the blood-cell types, insofar as they could be identified in the glycogen-stained smears. For example, glycogen appeared in some of the cells here named "nematocytes" (pl. 8, *D*, *c*) and "polypodocytes" (pl. 13, *B*, *g*). Certain types of blood cells, among which were the oenocytoids (pl. 5, *C*, *l*, and *D*, *f*), were usually found to show no glycogen.

Glycogen inclusions have been observed in mitotically dividing blood cells, apparently distributed to the two prospective daughter cells in unequal amounts. Glycogen was found also in some proleucocytes.

The smeared midgut epithelial cells were frequently recognized as being either columnar cells or goblet cells (*114*). Glycogen was observed in many but not in all of the columnar cells (pls. 3, *A* and *B*; 4, *A*–*C*; 6) in a given smear.

INTRACELLULAR LOCATION AND APPEARANCE OF GLYCOGEN IN BLOOD AND MIDGUT EPITHELIAL CELLS

The glycogen inclusions of the blood cells appeared in the form of small granules, rounded masses of various sizes and shapes, and irregular masses of different sizes (pls. 1, 2, and 5). In certain cells from glucose-fed larvae the cytoplasm had a more or less diffuse glycogen stain, and such cells often contained more deeply stained inclusions. Glycogen inclusions were not observed in the nuclei of the blood cells.

The glycogen inclusions in plasmatocytes from insects reared on turnip leaves usually consisted of one or two round to ovoid masses sometimes located one at each pole of the cell (pl. 1, *E*, and 2, *A*). They were similar in location, size, and shape to the large, colorless polar vacuoles in the plasmatocytes of Wright-stained smears (pl. 7, *C*). Sometimes a number of glycogen inclusions were observed in a single cell (pl. 1, *B*–*E*; 2, *C*), particularly in the prepupa. Greater numbers were observed in some of the plasmatocytes from the glucose-fed larvae, appearing as rounded, ovoid, or somewhat irregular masses varying from the size of a granule to that of a nucleus. An inclusion occurring singly in a cell was also observed to lie anywhere in this range, although usually it was larger than a granule (pl. 5, *C* and *D*).

The plasmatocytes sometimes contained glycogen inclusions in which the glycogen reaction appeared faint. Sometimes the red color (Bauer's stain) was localized at the periphery of the inclusion, the center appearing pale or colorless and the entire inclusion having a vacuolar aspect.

The glycogen inclusions of the cystocytes also appeared to vary with the glucose intake. Frequently blood smears contained glycogen in the plasmatocytes, though practically none were observed in the cystocytes. When glycogen inclusions were observed in the cystocytes, they usually appeared as several small granules distributed irregularly in the cytoplasm, as many small granules scattered throughout the cytoplasm, or as some condition of granule occurrence between these two extremes (pls. 2, *C*; 5, *C* and *D*). Sometimes cystocytes containing a number of the small glycogen granules also contained one or more larger, irregular glycogen inclusions (pl. 5, *C* and *D*).

In the smeared columnar epithelial cells of the midgut, glycogen inclusions appeared sometimes as irregular patches between the nucleus and the end of the cell (pls. 4, *A* and *C*; 6), at other times as masses located in other regions of the cytoplasm. These glycogen inclusions were frequently elongated in the direction of the long axis of the cell. No glycogen was observed in the nuclei of these cells.

THE GLYCOGEN COUNT AND THE GLYCOGEN INDEX

Glycogen counts were made from a number of blood smears obtained from larvae fed turnip leaves, collard leaves, and turnip leaf-cornstarch sandwiches containing different amounts of glucose. The larvae were fed for different lengths of time. Some of these larvae served as nonpoisoned controls in experiments on the effects of poisons on blood-cell glycogen. Some of the control larvae were ligatured by means of a string tied tightly about the body to separate it into approximate anterior two-third and posterior one-third portions; these larvae yielded counts from the fore and hind parts of their ligatured bodies. The period between application of the ligature and sampling of the blood ranged from 1 to 29 hours. Other control larvae were left unligatured. Under these different conditions the blood cells were found to contain various amounts of glycogen. The glycogen counts, however, exhibited similar general characteristics.

The fundamental form of the glycogen count is shown graphically in figure 1, *A*, in which the plotted points are averages of 94 counts from 73 larvae (21 ligatured plus 52 nonligatured) used as controls. This graph shows that on an average, even though counts from larvae fed large amounts of glucose were included, most of the blood cells contained no glycogen inclusions, some contained one inclusion, fewer contained two and still fewer three inclusions, and more cells contained over three inclusions than contained three. The glycogen count thus consists of the following classes: 0, 1, 2, 3, and 3+. The graph represents the general form of the majority of the individual counts. As shown by the glycogen index, the average larva of this group had glycogen in about 31 percent of its cells. In a few instances the 3+ class contained fewer cells than the 3 class. Examination of the corresponding blood smears showed that the cystocytes contained little or no glycogen.

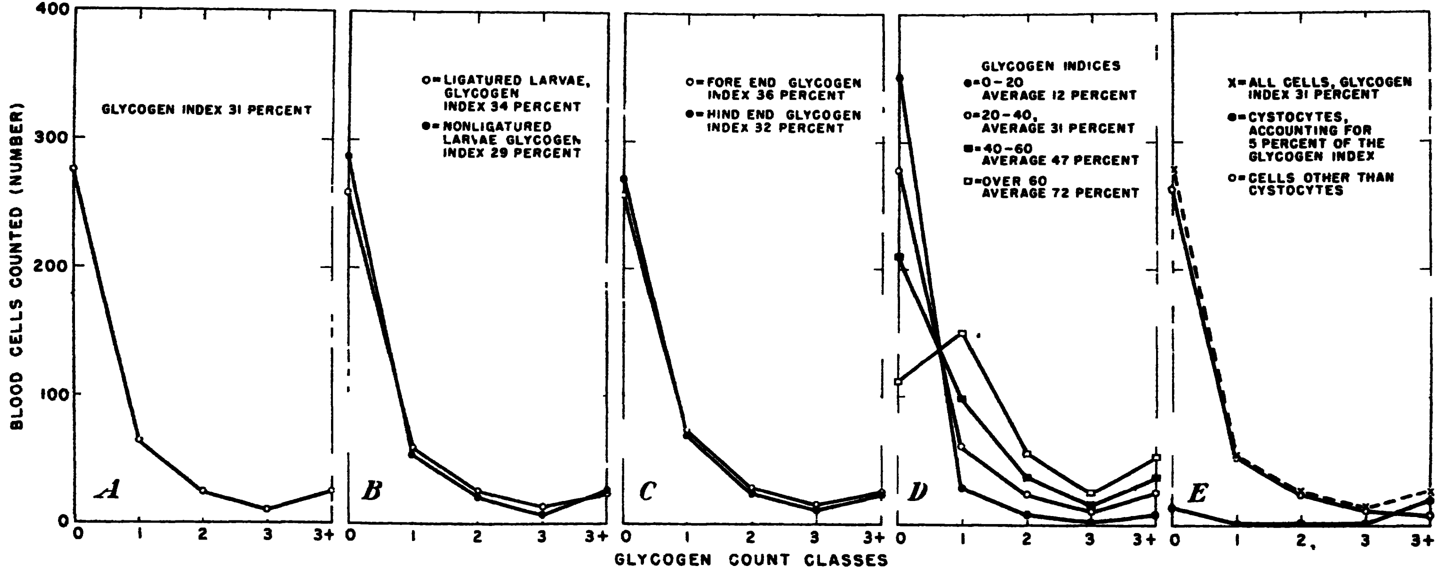


FIGURE 1.—Average glycogen counts and indices for larvae of *Prodenia eridania*: *A*, For 21 ligatured plus 52 nonligatured larvae; *B*, for 21 ligatured and for 52 nonligatured larvae, *C*, for the fore and the hind ends of ligatured larvae; *D*, for ligatured plus nonligatured larvae used as controls, grouped according to four glycogen index ranges, *E*, for 11 larvae fed turnip leaves, showing separation of cystocytes from other blood cells.

Figure 1, *B*, represents the average glycogen counts for the 21 ligatured and the 52 nonligatured larvae. The two curves are of the form shown in *A*, and differ chiefly in that they have different glycogen indices.

Figure 1, *C*, shows the average glycogen counts from the fore and hind parts of the 21 ligatured larvae. The average fore and hind end values are so similar that they might be represented by a single curve. The average glycogen indices are nearly the same.

In figure 1, *D*, are glycogen counts grouped according to the size of their corresponding glycogen indices. The curves show the way the glycogen count changes with change in index. As the glycogen index increases, the number of cells decreases in class 0 and increases in the other classes, as must happen; furthermore, the cells in class 1 become relatively more numerous than the cells in class 2, which show greater relative increase than the cells in class 3, and class 3+ undergoes a greater relative increase than class 3. When the glycogen index exceeds a certain value (60-70 percent in these experiments), the peak of the curve passes from class 0 to class 1.

Figure 1, *E*, shows the average glycogen count for 11 turnip-leaf-fed larvae. This curve has the general form already indicated in *A-D*. Other curves show the effect of counting separately the cystocytes and the cells other than cystocytes. When blood cells exclusive of the cystocytes were counted, the cells of class 3+ were not more but less numerous than those of class 3, the cystocytes were more numerous in class 3+ than in class 3 or classes 2 and 1, and in class 3+ the cystocytes outnumbered the other cells.

Figure 2 shows the frequency distribution of the glycogen indices of the 21 ligatured larvae (fore and hind ends), the 52 nonligatured larvae, and the 73 ligatured plus nonligatured larvae used as controls, with regard to their respective averages of 34, 29, and 31 percent.

Figure 3 shows average glycogen indices of the second to sixth instars, and of the prepupal and pupal stages. The averages are based upon determinations from 5 second, 6 third, 3 fourth, 8 fifth, and 11 sixth instars, 6 prepupae, 11 pupae 1 day old, 3 pupae 2 days old, 2 pupae 3 days old, and 4 pupae 10 days old. Although the number of insects used was not great, figure 3 indicates the manner in which the glycogen index varied with stage of larval development and with metamorphosis. The average index for the sixth instar was 31 percent, and for the prepupa 35 percent. Glycogen was not found in larvae at the time of hatching; therefore the glycogen index was 0 at that time. During larval development the glycogen index increased until it attained a maximum in the prepupa. It rapidly decreased to a very low value during the first few days after pupation, and continued low throughout the rest of pupal life. The few incidental observations made on the adults indicated that the glycogen index of the adult tended to be very low.

POLYSACCHARIDE IN OTHER TISSUES

Incidental observations were made of a polysaccharide, stained by the Bauer technique, in cells other than those of blood and midgut epithelium. Since the Bauer method gives positive results with polysaccharides other than glycogen, and since only the Bauer method,

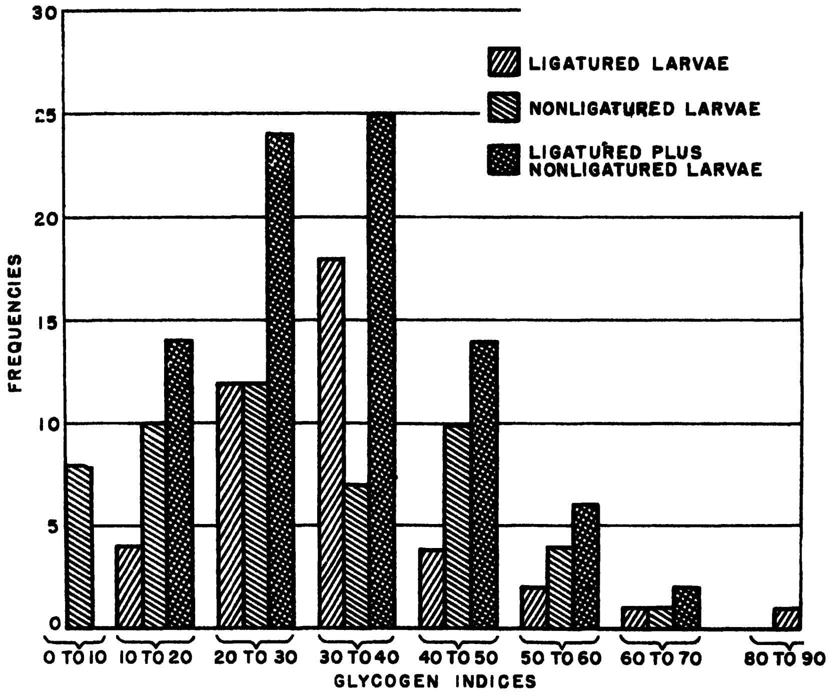


FIGURE 2.—Histogram showing the frequency distribution of 94 glycogen indices from fore ends and hind ends of 21 ligatured larvae, from 52 nonligatured larvae, and from the total 73 ligatured plus nonligatured larvae of *Prodenia eridania* used as controls.

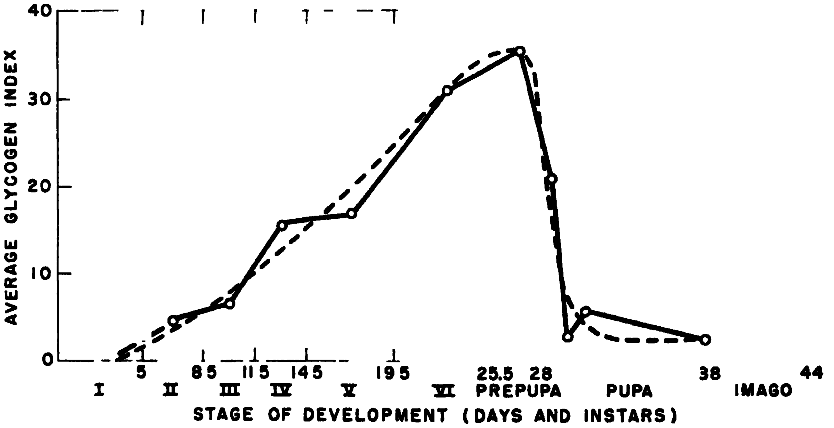


FIGURE 3.—Change of average glycogen index with development of *Prodenia eridania*. Along the abscissa roman numerals represent instars and arabic numerals represent days after hatching. The broken-line curve is hypothetical.

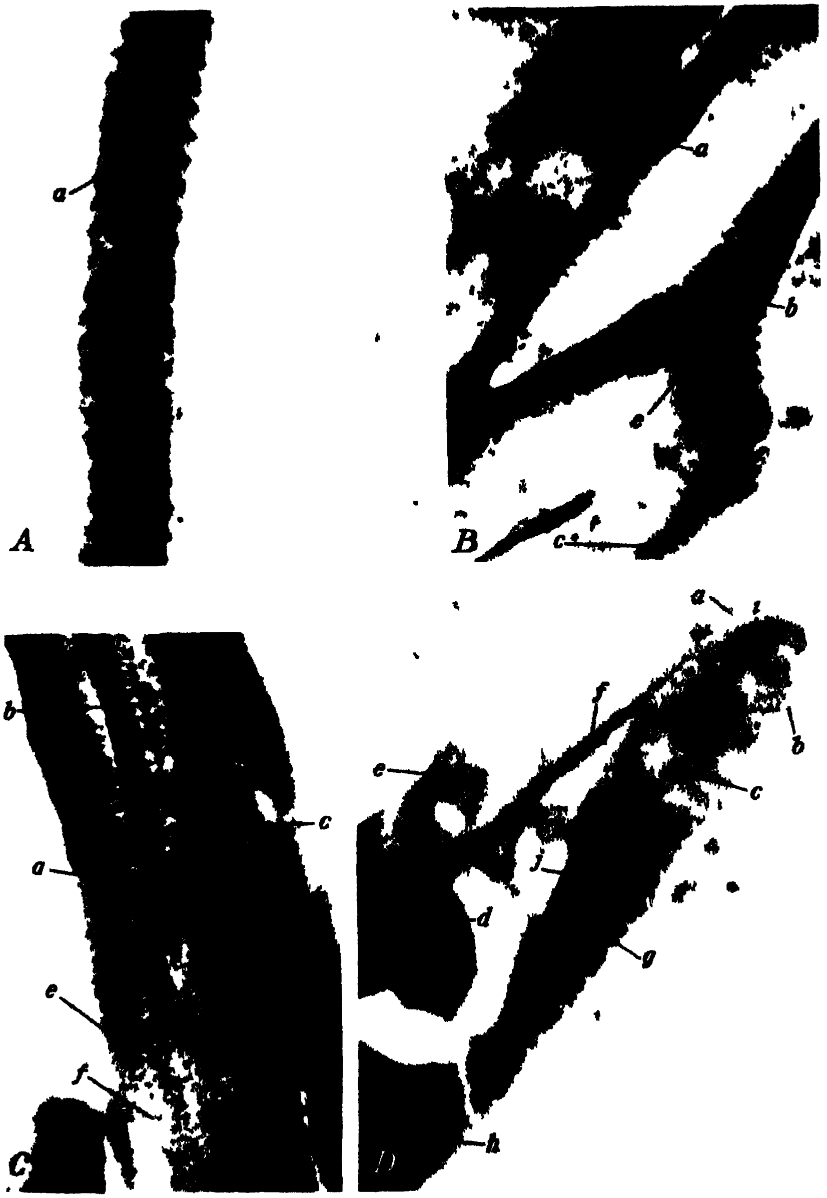
without counterproof, was applied to most of these tissues, the broader term "polysaccharide" is here used except in those cases where the Lugol stain was also applied.

The polysaccharide was detected in the following tissues of a sixth instar that had been fed overnight on a turnip leaf-cornstarch-glucose sandwich, and whose blood-cell glycogen index was 35 percent: Ganglia of the ventral nerve cord, labial glands, Malpighian tubules, fat body, oenocytes, testes, pericardial cells, integument, walls of the foregut and hindgut, midgut epithelial cells, and striated fibers of the body muscles, the gut musculature, the cardiac muscle, and the alary muscles. It was not determined whether the polysaccharide in the ventral nerve ganglia and the interganglionic connectives (pl. 10, *A*) was localized in nerve tissue or in associated nonnerve tissue. The polysaccharide inclusions of the labial-gland cells (pl. 10, *B*) appeared to be numerous, irregular masses of various sizes, many of a coarse granular aspect. The walls of the Malpighian tubes (pl. 10, *C*) contained numerous coarsely granular polysaccharide inclusions, whose relation to the tubular cells was not definitely determined. Similarly, the cytological location of the numerous polysaccharide inclusions of the fat body (pl. 10, *D*) was not definitely determined. The polysaccharide of the striated muscle fibers of the body (pl. 11, *A*), of the fore, mid, and hind parts of the gut (pl. 11, *B*), and of the cardiac muscle (pl. 11, *C*) appeared to occur diffusely, but in addition appeared particularly concentrated at the cross striations, which were seen as deeply stained double lines. The polysaccharide of the alary muscle fibers (pl. 11, *D*) appeared slight but also associated with the cross striations. The oenocytes were opaque, but in places their cytoplasm appeared to be filled with granular polysaccharide inclusions of various sizes and shapes. Polysaccharide was associated with the testes (pl. 10, *E*), but the kinds of sells containing it were not identified; it was not detected in several stages of developing germ cells that were examined. The cytoplasm of the pericardial cells (pl. 11, *D*), contained many very small, granular polysaccharide inclusions. Polysaccharide was also found in the integument, but its cytological location was not determined. The sclerotic membrane of the integument, as well as the sclerotic linings of the fore- and hind-guts, gave a positive reaction with the Bauer technique. Polysaccharide inclusions were not observed in those nuclei whose structure could be sufficiently examined. In the smears of the fat body some of the polysaccharide inclusions were associated with definite masses of basophilic protoplasm (pl. 10, *D*), but whether with nucleoplasm was not determined.

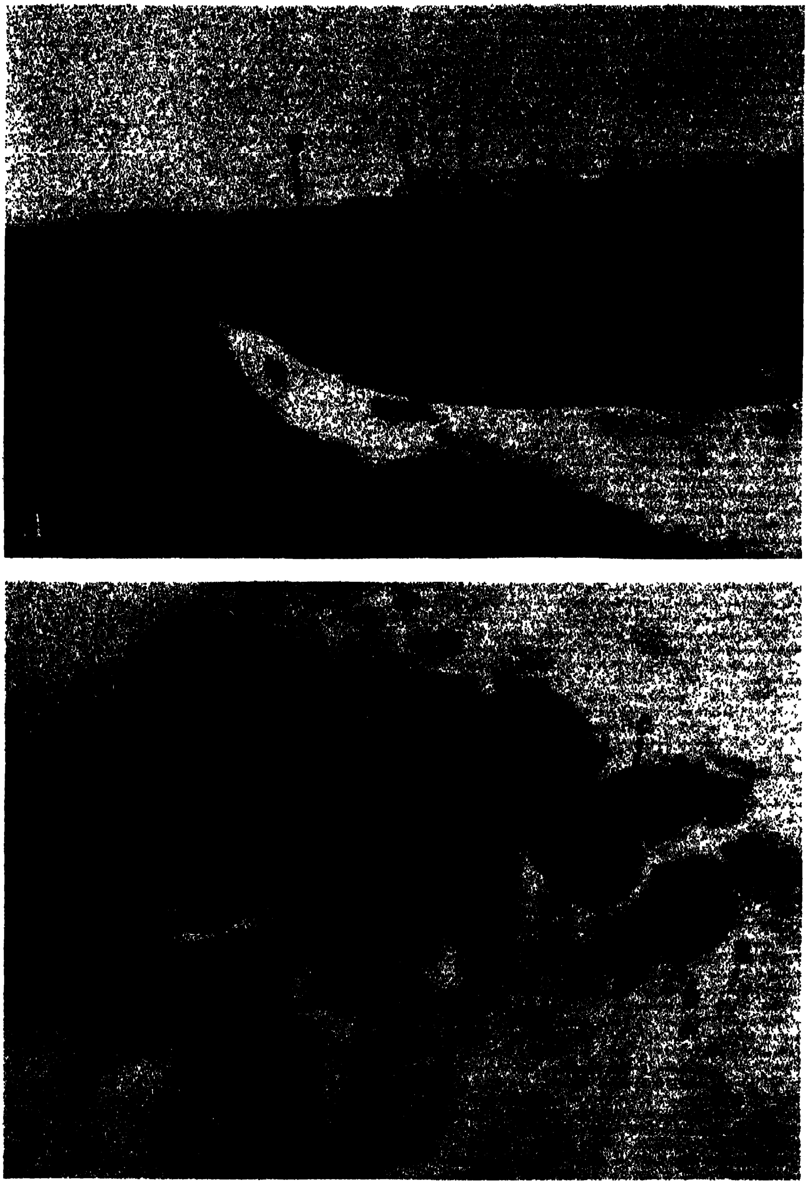
In some of the early instars polysaccharide was found incidentally in the Malpighian tubes, labial glands (pl. 12, *A*), and oenocytes (pl. 12, *B*), when Bauer's technique was employed, and in the labial glands and oenocytes when they were stained with Lugol's solution.

EFFECTS OF DIFFERENT FIXING AND STAINING METHODS

Although at least traces of glycogen were detected histochemically with nearly all the fixatives mentioned under Methods, preliminary experiments with different fixatives and with the Best and the Lugol staining procedures led to the conclusion that the most satisfactory fixations were obtained with Carnoy's fluid and with absolute alcohol. This is indicated for the Lugol procedure by the glycogen indices given in table 1, which were obtained from two larvae that had fed overnight on glucose-cornstarch-turnip leaf sandwiches.



Different kinds of muscle fibers from smears of a sixth instar *Prodenia eridania* that had been fed turnip leaf cornstarch glucose sandwich. *A*, Part of body muscle with cross striations as at *a*; *B*, branched muscle fibers of the foregut, as at *a*, *b* and *c* showing cross striations, as at *c*, and bacteria from gut contents as at *d*; *C*, cardiac muscle with cross striations, as at *a*–*d*, and fiber boundaries as at *e* and *f*; *D*, alary muscle fibers, *a* and *b*, with cross striations faintly visible, as at *f*, *g* and *h*, pericardial cells, *c*, and cardiac muscle, *d*, with cross striations visible at *e*. Polysaccharide appears diffusely throughout the muscle and muscle fibers and is especially concentrated at the cross striations; in the pericardial cells it appears as small granules distributed in the cytoplasm, which accounts for the dark areas *i* and *j* in *D*. Bauer technique, counterstained with thionin dye, 10X ocular, 45X objective.



Tissue smears from a 24-hour-old first-instar *Prodenia eridania* fed turnip leaves, showing polysaccharide inclusions: *A*, In cells of a labial gland, *d*, at *a*, *b*, and *c*; *B*, in some faintly stained oenocytes at *a*, *b*, *c*, and *d*. Bauer technique, counterstained with thionin; dry; 10× ocular, 45× objective.

Glycogen indices from Bauer-stained smears of these larvae were 35 percent for larva 1 and 25 percent for larva 2.

TABLE 1.—*Adequacy of different fixatives when used with the Lugol procedure, as indicated by the glycogen index*[1]

Fixative	Larva 1		Larva 2		Remarks
	Time in fixative	Glycogen index	Time in fixative	Glycogen index	
	Hours	*Percent*	*Hours*	*Percent*	
Absolute alcohol (2 changes)	26	35.75	29	29.25	Cells shrunken, glycogen inclusions distinct
Carnoy (with chloroform)	4	35.00	5	28.00	Cells well fixed, most glycogen inclusions distinct but some so faint as to be questionable
	26¼	39.00	28¼	33.50	
Neukirch (formol saturated with glucose)	26	3.00	29		Cells somewhat distorted, glycogen inclusions faint
Zenker	27	23.50	27	23.50	Cells shrunken, glycogen inclusions faint but well defined
Bouin	27½		26½		Vacuoles distinct but colorless
Pasteels and Léonard	1	8.00	1¼	18.25	Cells distorted, glycogen inclusions when present, generally faint
	4½	25.25	4¾	1.50	
	27	13.00	28		
Bouin-Allen	4¾	6.75	5½	6.25	Vacuoles distinct but nearly colorless
	27		26		
Vastarini-Cresi	26		28	11.50	Cell fixation fair, glycogen inclusions indistinct and faintly stained
Gendre	1¼	2.75	4	5.50	Cells distorted, glycogen inclusions, when present, faintly stained
	27		28		

[1] No indices were obtained from the smears with extremely poor glycogen fixation.

The glycogen inclusions of the blood cells and midgut epithelial cells were more clearly defined in the thionin-counterstained Bauer smears than in the wet Lugol preparations. Chromic acid fixation involved some shrinkage of either cells or plasma film, or both, as indicated by a space between cell and plasma (pls. 2, *C*; 5, *C* and *D*). Although the thionin counterstain persisted for months, eventually it faded. None of the saliva-treated smears contained glycogen, and none of the smears treated according to the mucicarmine method of Mayer gave positive results, although they were from larva that contained blood-cell glycogen as indicated by the Bauer reaction.

Plates 13, *A*, and 14, *A* and *B*, illustrate the results obtained by combining Best's carmine and the Wright techniques. In cells thus stained the eosinophilic nuclei and basophilic cytoplasms were stained as with the Wright procedure alone and the glycogen inclusions were stained a dark blue. Plate 13, *B*, shows cells stained with Best's carmine and counterstained with Ehrlich's haematoxylin. The glycogen inclusions are stained the usual carmine color.

DISCUSSION

OCCURRENCE OF BLOOD-CELL GLYCOGEN IN ANIMALS

The results described above show that the appearance of glycogen in the blood cells of *Prodenia eridania* is a normal occurrence. The question naturally arises as to whether the southern armyworm is exceptional in this respect or whether such occurrence is general among animals. The authors have found in the literature no paper on insect blood-cell glycogen and only a few papers that deal incidentally with its histochemical detection. Vaney and Maignon (*104*), using the gum-iodine method and the gentian violet method of Lubarsch, reported the histochemical detection of glycogen in leucocytes found associated with adipose cells in the silkworm during metamorphosis; they consider the glycogen not to be of histolytic origin. Rudolfs (*96*), also using iodine and gentian violet staining methods, reported the detection of glycogen in the leucocytes of the adult tent catapillar (*Malacosoma americana* (F.)). De Boissezon (*16*) reported that, using the gum-iodine and Best's carmine methods, he found no glycogen in the amoebocytes of the mosquito *Culex pipiens* L., and Paillot (*79*), using Gendre's method of fixation and the staining methods of Best and Lugol, reported no detectable glycogen in the blood cells of the silkworm.[5] Bogojavlensky (*14*), using Carnoy's fixative and the Best carmine and the Lugol iodine staining methods, found glycogen in the spindle-shaped leucocytes and the proleucocytes of *Carausius morosus* Brunner and, by implication, in seven other species of Orthoptera that were used for comparison; he also found a little glycogen in small, round blood cells, but not in the proleucocytes and spherocytes, of the silkworm and, by implication, of nine other species of Lepidoptera. Ronzoni and Bishop (*93*), using chemical methods, found that in the mature feeding and the spinning honeybee larvae the concentration of blood glycogen was less than 10 mg. percent—i. e., not detectable—and that blood glycogen appeared in greater concentration—i. e., became detectable—only at a later stage, when the cells of the fat body broke down during metamorphosis. Their work did not indicate whether the glycogen occurred in blood cells. Roy (*95*) failed to detect glycogen in the blood of the larva of the wax moth (*Galleria mellonella* (L.)).

Babers (*3*) found that the normal glycogen content of the blood of the sixth-instar southern armyworm was from 1.3 to 4.5 mg. per 100 ml., with an average of approximately 3.3 mg., and that the glycogen content of pooled blood from larvae that had fed overnight on glucose ranged from approximately 13.5 to 33.4 mg. per 100 ml. In one experiment Babers found that a blood sample contained 17.42 mg. of glycogen per 100 ml., of which 16.5 mg. was in the centrifuged cells and only 0.92 in the supernatant plasma, and he suggested that the plasma glycogen may have come from some cells ruptured by centrifuging. Babers' glycogen values were obtained from the blood of insects that had been immersed in water at 60° C. for 1 minute. Thus, blood-cell glycogen is reported to occur in the silkworm, the tent caterpillar, the southern armyworm, *Carausius morosus*, and according to the implications of Bogojavlensky's paper, in a number of other species of Orthoptera and Lepidoptera.

Blood-cell glycogen has also been reported in invertebrates other than insects. Barfurth (*5*) suggested that it probably occurs in gastropods as well as in Crustacea. Creighton (*22*) found glycogen in the blood cells of snails. Biedermann (*12*) stated that glycogen occurs in annelid leucocytes. Its occurrence in various protozoa is indicated by Pflüger (*83*) and Biedermann (*12*), who refer to other literature, and by Giovannola (*41*) and Creighton (*22*).

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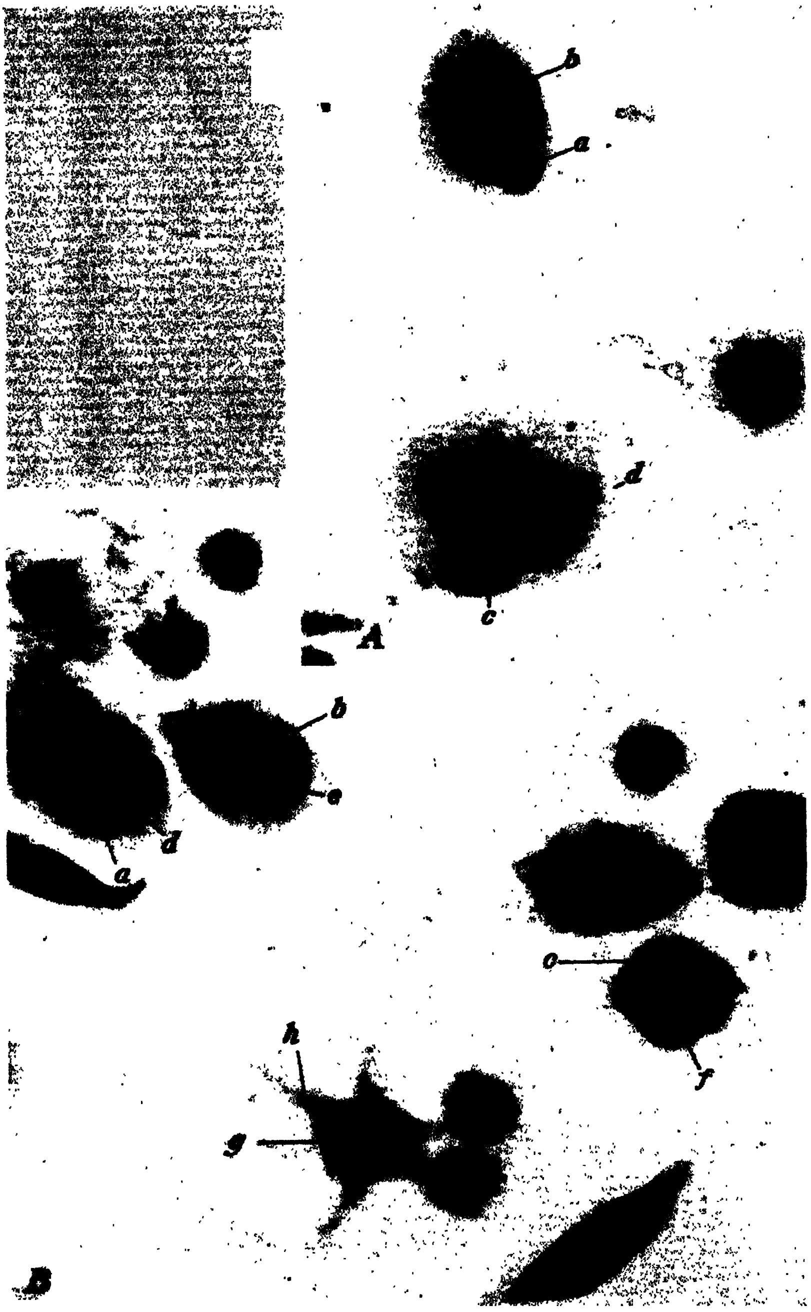
[5] The present authors found inclusions of Bauer-positive polysaccharide in the blood cells of 3- or 4 day-old first-instar silkworms that had been fed only lettuce leaves. These polysaccharide inclusions appeared similar to the glycogen inclusions of southern armyworm blood cells—i. e., in plasmatocytes—and no doubt were glycogen inclusions. However blood cells of silkworms that were reared on mulberry leaves and were nearing the end of their larval life showed no positive reaction to the Bauer technique.



Blood cells from a sixth-instar *Prodenia eridania* reared on turnip leaves: *A*, Blood smear stained by combined Best's and Wright's techniques; *B*, blood smear stained by Best's method and counterstained with Ehrlich's haematoxylin. Large glycogen inclusions in *A* are shown at *a* in plasmatocyte *b*, and at *c* and elsewhere in plasmatocyte *d*; in *B* in plasmatocytes *a*, *b*, and *c*, at *d*, *e*, and *f*, respectively; *g* is a polypodocyte, with pseudopodlike extensions of the cytoplasm, as at *h*. Oil immersion; 10× ocular, 93× objective.

A, Plasmatocyte from a blood smear of a newly formed pupa, and *B*, midgut cells from a tissue smear of a second instar of *Prodenia eridania*, both stained by combined Best's and Wright's techniques *A* shows a large glycogen inclusion, *a*, and several small inclusions, as at *b*, in *B* glycogen inclusions appear as black masses, as at *a*–*f* *A*, Oil immersion, 10× ocular, 93× objective; *B* dry, 10× ocular, 45× objective

Most of the literature on the occurrence of blood-cell glycogen of vertebrates appears to have reference to mammals, particularly to man.[6] Ranvier (*89*) found glycogen in frog leucocytes and, according to Giradin (*42*), was the first investigator to demonstrate glycogen in leucocytes, but Schäfer (*98*) also early referred to glycogen in leucocytes, presumably of man. Barfurth (*5*) failed to find glycogen in either cells or plasma of frog blood and did not obtain decisive results on human blood, but he reported blood-cell glycogen in vertebrate leucocytes. Frerichs (*36*), Gabritschewsky (*38*), Dastre (*23*), Kaminer (*56*), Bakker (*4*), Willstätter and Rohdewald (*113*), Fieschi and Bertola (*33*), Giradin (*42*), Golandas (*43*), Staub and Golandas (*100*), Wallbach (*108*), Unshelm (*103*), Kotschneff (*61*), Havet (*47*), Howell (*54, p. 881*), Downey (*29*), Karsner (*57, pp. 61–63*), and Cowdry (*21*) are some authors who state that glycogen occurs normally in certain leucocytes of man and other mammals (dog, cat, calf, herbivores, horse, rabbit). It would seem that usually glycogen occurs chiefly in the granulocytes, especially the neutrophiles, and to a slight degree or not at all in the lymphocytes and monocytes; see, for example, Giradin (*42*), Downey (*29*), Cowdry (*21*).

According to Dastre (*23*), glycogen was first observed microscopically in pus cells by Kühne (1865) and was shown by other investigators, whose papers Dastre cites, to become more evident microchemically under certain conditions of disease. Fieschi and Bertola (*33*), Giradin (*42*), Unshelm (*103*), and Karsner (*57, pp. 61–63*) also consider leucocytic glycogen in pathological conditions, primarily in the blood of man.

It thus appears from the literature that blood-cell glycogen occurs among protozoa, annelids, mollusks, crustaceans, insects, amphibia, and mammals, including man. Although the observations on mollusks and crustaceans seem to be very few, it is probable that blood-cell glycogen is of general occurrence among animals. This conclusion is in accordance with the concept expressed by Bernard (*8*), Barfurth (*5*), and Dastre (*23*) that glycogen may occur in all or most tissues of all or most animals. The southern armyworm, therefore, is not exceptional in containing glycogen in its circulating blood cells.

INFLUENCE OF NUTRITIONAL CONDITIONS ON BLOOD-CELL GLYCOGEN

The results of the experiments with newly hatched southern armyworm larvae indicate that the appearance of both blood-cell and gut-cell glycogen is dependent on food intake, particularly that of carbohydrate. With sixth instars the amount contained in the blood cells varies with nutritional conditions, especially the amount of carbohydrate ingested. Thus, prolonged starvation may cause the disappearance of blood-cell glycogen, and the ingestion of relatively large quantities of glucose may cause glycogen inclusions to appear in a large percentage of the larva's blood cells.

Such dependence of blood-cell glycogen on carbohydrate intake may help to explain negative results such as those reported by de Boissezon (*16*) and Paillot (*79*), as well as the marked individual differences in both blood-cell and gut-cell glycogen that were noted in the present investigation. Harnisch (*46*) found no evidence of glycogen resynthesis in *Chironomus* larvae during post-anaerobic recovery, except through the ingestion of food. Kato (*58*) found that more glycogen appeared in silkworm larvae fed immature mulberry leaves than in larvae fed mature leaves. Kato, Miwa, and Negi (*59*) obtained glycogen increase in silkworm larvae upon adding sugar to their diet. Kuwana (*63*) found that glucose ingestion caused an increase in total reducing power of the blood in silkworm larvae and considered an increase of glycogen or of a carbohydrate-protein complex to be involved. Thus, it may be expected that, other conditions remaining the same, an increase in the carbohydrate content of the leaves upon which the larva feeds will result in an increase in insect blood-cell glycogen.

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[6] A number of the references dealing with glycogen in vertebrate blood cells were kindly supplied by C. N. H. Long, of Yale University.

PROBABLE RELATION OF BLOOD-CELL GLYCOGEN TO BLOOD SUGAR IN INSECTS

Babers (*3*) found that the ingestion by the sixth-instar southern armyworm of turnip leaf smeared with glucose-cornstarch paste was followed quickly by a sudden and relatively large rise in blood sugar, which reached a maximum, then quickly fell, and returned to the normal value in approximately 6 hours. He also found that tissue (exclusive of blood and gut) glycogen also quickly began to rise, but rose more slowly, reached a maximum considerably later, and then descended to, not the initial, but a somewhat higher tissue-glycogen level. He further found that a rise in blood glycogen occurred still later, appearing only after a relatively long period of glucose ingestion, and that practically all the blood glycogen was associated with the centrifuged blood cells.

The histochemical observations of blood-cell glycogen reported in this paper are in agreement with the findings of Babers. It is quite probable that Babers' average glycogen value for normal blood of 3.29 mg. per 100 ml. corresponds to the glycogen index of approximately 31 percent reported here for sixth instars fed collard and turnip leaves; but, whereas Babers obtained a blood-glycogen increase of sixfold to tenfold after overnight feeding of a glucose-starch-turnip leaf diet, the highest glycogen index obtained in the present experiment was about 86 percent, representing only about a threefold increase, under fairly similar conditions. Although this difference might be due to differences in food intake, it might be explained by the fact that the glycogen count and the glycogen index are based, not on the amount of glycogen per cell, but on the number of glycogen inclusions per blood cell, and that microscopic observations show that after ingestion of the carbohydrate diet many of the blood cells contain relatively large glycogen inclusions. The glycogen counts and indices reported here also show that, as the glycogen index increases, more cells enter classes 3 and 3+ of the glycogen count. Thus, it seems that an increase of blood-cell glycogen involves an increase in the number of cells containing glycogen, in the amount of glycogen contained within at least some of the glycogen inclusions, and in the number of glycogen inclusions per cell. It is also possible that glycogen not detectable histochemically may occur and increase with the carbohydrate intake.

Baber's suggestion that the delayed rise of blood glycogen might be associated with a tendency to rise only after the other tissues had become saturated with glycogen receives support from the present observations on glycogen in the tissues of a sixth instar that had fed overnight on a turnip leaf-cornstarch-glucose sandwich. The glycogen index of this larva was about 35 percent, and its tissue cells, particularly those of its labial gland, Malpighian tubes, striated muscles, pericardial cells, oenocytes, and fat body, had the aspect of being heavily loaded with polysaccharide, as indicated by the Bauer technique. The observations on the newly hatched larvae, which showed that blood-cell glycogen first appears later than gut-cell glycogen, also are consistent with the suggestion mentioned above. Thus, a marked or persistent exogenous hyperglycemia seems to produce an increase in both chemically and histochemically detectable blood-cell glycogen, and, on the assumption that starvation results in a lowering of blood sugar, the results of the starvation experiments imply the existence of an analogous relationship between a marked or persistent starvation hypoglycemia and a decrease of blood-cell glycogen. There is some evidence, as reported, for example, by Staub and Golandas (*100*), that administration of glucose to the mammal causes blood-cell glycogen, but not plasma glycogen, to increase. That a lowering of insect blood sugar might result from starvation is indicated in the literature; see, for example, Sturtevant (*102*), Beutler (*11*), and May (*72*). Demjanowski and Prokofjewa (*26*) reported that the total reducing power of silkworm blood was not dependent on the quantity and quality of the food given the insects. Hemmingsen (*48*) reported a decrease in total reducing power of the blood of several species of insects as a result of starvation, but he considered the decrease not to involve blood sugar. That glucose accounts for only a small part of the total reducing power of insect blood is indicated by Hemmingsen (*48*), Kuwana (*63*), Florkin (*34*), and Babers (*2*), as well as others.

Most of the literature having to do with the relation between sugar and glycogen in insects is concerned with the concentrations of these carbohydrates, not in the blood, but in the whole insect, and will be found summarized by Babers (*3*). The observations and results reported in that literature and those reported here indicate that under certain conditions, chiefly in the pupa, the glycogen content of the insect, including blood-cell glycogen, can decrease in the presence of an elevated blood sugar. This is not contradictory to the statement made above that an exogenous hyperglycemia tends to increase the glycogen content of the blood cells, for the elevated blood sugar of metamorphosis may be considered to be endogenous and to involve other metabolic conditions.

It thus appears that the insect, like the vertebrate, possesses a physiological mechanism whereby gluconeogenesis occurs in the blood cells during alimentary hyperglycemia, and that in insects glycogenolysis occurs during presumptive starvation hypoglycemia and during the endogenous hyperglycemia of metamorphosis.

OTHER FACTORS THAT MIGHT AFFECT THE OCCURRENCE OF BLOOD-CELL GLYCOGEN

FOOD FACTORS

In this investigation it was found that when larvae were reared or fed for a sufficiently long time on white cabbage, blood-cell glycogen was not readily demonstrable, many of the larvae apparently having none. MacDonald and Wislicki (*69*) prepared two cabbage extracts that they found affected the blood sugar and liver glycogen when fed to mammals. Their hyperglycemic fraction caused a marked increase in blood sugar in the rabbit, accompanied by glycosuria and a decrease in the glycogen content of the liver. Their hypoglycemic fraction "vegulin" lowered the blood sugar and appeared successfully to replace insulin in the depancreatized dog. It is not impossible that such food factors may affect feeding insects, and that such a hyperglycemic food factor in the cabbage may have been partly or wholly responsible for the relatively slight blood-cell glycogen observed in the cabbage-fed larvae of the experiments reported here. Braun and Rees (*18*), in a review of literature pertinent to this subject, indicate that the question of the occurrence of insulinlike substances in plant extracts is in a very unsettled state.

HYDROGEN-ION CONCENTRATION

Babers (*2*) found the pH values of the blood of sixth-instar southern armyworms to range from 6.40 to 6.67, with an average of 6.53. Demjanowski, Galzowa, and Roshdestwenska (*25*) found the hydrogen-ion concentration of the blood of silkworm larvae to decrease at the molts, and to be slightly but persistently higher in the female than in the male. Vaney and Maignon (*104*, *105*) reported that the glycogen content of *Bombyx mori* L. was greater in the female than in the male. Babers (*3*) found the glycogen content of the southern armyworm adult to be persistently higher in the female than in the male. Bernard (*9*) reported that the larva of a fly showed much glycogen, no sugar, and an alkaline reaction of the tissues, the pupa showed sugar and an acid reaction of the tissues, and the adult continued to show both sugar and glycogen. Bernard also reported that the liver glycogen of Crustacea increases at the molt and decreases or disappears between molts. Elias and Weiss (*30*) reported that glycogen in frog eggs decreased when the pH value of the suspension medium dropped. Donnelly (*28*) discussed generally the question of hydrogen-ion concentration and glucose metabolism and concluded that glycogenolysis varies directly with the total acidity of a single tissue or of a whole organism. Other investigators have reported observations that have a bearing on the subject. The hydrogen-ion concentration of the blood, therefore, may be an influential factor in the occurrence of blood-cell glycogen.

METABOLIC CONDITIONS

A number of poorly understood or unrecognized factors that influence the presence of blood-cell glycogen may exist in the insect either continuously or under certain conditions of metabolism. Maignon (*70*) and Vaney and Maignon (*104*) produced asphyxia in *Bombyx mori* by immersing the insect or its tissues in boiled oil and irregularly obtained increases of body sugar. Lesser (*66*) stated that glycogen of the frog is strongly hydrolyzed as a result of anoxybiosis.

Harnisch (*46*) obtained an anaerobic decrease of glycogen in certain tissues of *Chironomus* larvae. Rudolfs (*96*) stated, but gave no supporting data or literature reference, that anesthesia of a living cell inhibits the synthesis of glycogen. That the local metabolic processes in a cell or tissue may influence the amount and the character of the glycogen reserve is indicated by the observation of Bogojavlensky (*15*) that the external lobes of the fat body of the silkworm contained more histochemically detected (Best method) glycogen than did the internal lobes, and that the glycogen of the fat body was less soluble in water than was the glycogen of other tissues. The same thing is indicated by the fact that the vertebrate neutrophiles, but usually not the mononuclears, contain considerable glycogen (*4*, *21*, *29*, *42*), and by the observations herein reported that in the normal southern armyworm the plasmatocytes and the cystocytes tended to exhibit glycogen inclusions more readily than did the other blood-cell types. It is generally recognized that muscular exercise causes a decrease of mammalian liver glycogen (*82*, *p. 94*), and Giradin (*42*) found that in man the number of circulating granular leucocytes that contain iodophilic substance increases at meal times and decreases during exercise after meals, tending to parallel the changes in blood-sugar concentration. As shown in the literature, some of which has already been cited, leucocytic glycogen occurs plentifully in pus cells and appears to vary with different diseases.

In the vertebrate fat metabolism and carbohydrate metabolism are closely related (*82*, *pp. 11, 13, etc.*). The literature reviewed by Babers (*3*) indicates the existence of some kind of an association between fat and carbohydrate metabolism in the insect. Fat inclusions in insect blood cells have been observed by a number of authors (Hollande *50*, Hufnagel *55*, and Rooseboom *93*), and possibly they occur in southern armyworm leucocytes, although in the present work no fat detection was attempted. It is therefore possible that in the insect blood-cell glycogen and blood-cell fat may be related. Karsner (*57*, *pp. 61–63*) states that glycogen is likely to appear in many places where fat occurs, and expresses the opinion that similar factors may influence both fat and glycogen. Insofar as they indicate an exogenous origin of blood-cell glycogen, the observations reported here seem to be in accordance with the view of Vaney and Maignon (*104*), who, disagreeing with Bataillon (*6*), considered that glycogen in the leucocytes and other cells of the metamorphosing silkworm is not of histolytic origin.

Cold tends to decrease liver glycogen in the mammal (*82*, *p. 24*), but it appears that the low temperature reflexly initiates muscular contractions (shivering) and that the glycogen decrease is thus related to exercise. If the armyworm possesses no mechanism analogous to the shivering reflex of the mammal, whereby muscular contractions are produced by cold, low temperature could be expected to tend to maintain its glycogen stores or to decrease their rate of disappearance, except insofar as it would tend to decrease the ingestion of food. Ditman and Weiland (*27*) have shown that a decrease of temperature retards the disappearance of glycogen in the pupa of the corn earworm. The honeybee, however, seems to possess a reflex mechanism whereby muscular contraction increases with decrease in hive temperature (*74*, *80*, *84*). Thus, in such an insect it is quite possible that cold would tend to cause a reflex lowering of any glycogen reserves, including blood-cell glycogen.

Bernard (*8*) early observed that in the higher animals glycogen is widely distributed in the tissues of the developing organism but becomes relatively localized in certain tissues of the adult, whereas in the invertebrates, which he considered to lack true livers, glycogen appears to be more widely distributed among the various tissues. Karsner (*57, pp. 61–63*), however, referring primarily to man, expressed the opinion that practically any cell in the body may contain glycogen although it may not be microscopically demonstrable. It thus seems that most of the tissues of animals can contain glycogen but differ chiefly with regard to the amount contained. From this standpoint it might be expected that glycogen would occur in at least some blood cells of various organisms, whether developing or mature.

NEURAL AND HORMONAL FACTORS

In the vertebrate storage and mobilization of liver glycogen are greatly influenced by neural and hormonal factors. It has been demonstrated (*82, p. 190; 99, p. 675;* and *10, p. 938*) that puncture of the floor of the fourth ventricle of the vertebrate brain causes a decrease of liver glycogen, and that both the nervous and the hormonal systems are operative in the process. The autonomic nervous system is considered to be primarily involved (*99, p. 675*). It is also generally recognized (*82, pp. 139–142; 44, p. 849; 99, p. 680; 71, p. 829; 10, pp. 924 and 938*) that, at least under certain conditions, insulin tends to increase and adrenalin to decrease the glycogen reserves of the liver. Arthropods, including insects, have long been known to possess a sympathetic or stomatogastric nervous system (*45, pp. 448 and 518*). Like the vertebrate autonomic nervous system, it seems to be especially associated in a functional manner with such autonomic processes as cardiac and perhaps respiratory rhythms, and may possibly be related, either directly or indirectly, to such metabolic processes as hormone secretion and storage and mobilization of energy reserves in the tissues. That the insect nervous system is related to hormone secretion is indicated by a number of investigations (*35, 60, 65, 109, 110, 111, p. 40,* and papers cited in *90*).

Medvédéva (*73*), determining total reducing power, reported that hyperglycemia was produced in the silkworm by injection of adrenalin and to a less extent by injection of water. She attributed the water hyperglycemia to a mechanical stimulation of the vegetative nervous system by the needle puncture. She found that, although the adrenalinic hyperglycemia could be produced in the fifth instar, it could not be produced in the spinning larva, the chrysalis (at about the middle of the pupal period), or the adult. She attributed these differences to changes in the nervous system of the spinning larva and the pupa, to some unknown factor that assures glycogen storage in the spinning larva, and to a lack of glycogen reserve in the adult. Medvédéva obtained no insulin hypoglycemia by injecting insulin into the silkworm larva and adult, but did obtain a hyperglycemia in the larva, which he ascribed to mechanical stimulation of the nervous system. Ermakov (*31*) obtained an increase and a decrease of reducing power in silkworm blood by ligaturing the body of the larva in different regions and removing that portion not used for determination of reducing power of the blood. He considered the hyperglycemia to be due to disturbance of the larva's sympathetic nervous system but had no explanation for the hypoglycemia. Hemmingsen (*49*) injected insulin into larvae but obtained such variable results that no conclusion could be reached regarding the effect of this hormone on the insect's blood sugar.

Thus, observations and results reported in the literature suggest that in the insect hormones occur in relation to the nervous system, and that the insect's physiological mechanism, whereby it stores, mobilizes, and controls its glycogen reserves, probably involves both neural and hormonal components. It is obvious, however, that neural components are not directly involved in a control of blood-cell glycogen, even though they should directly affect other glycogen-containing tissue cells. Although insufficient data are available to show whether the blood-cell glycogen is under hormonal control, the occurrence in insects of hormonal control of or influence on blood-cell glycogen is a possibility.

SIGNIFICANCE OF BLOOD-CELL GLYCOGEN IN THE DIFFERENT INSTARS

The results reported above and illustrated in figure 3 show that blood-cell glycogen increases during development to a maximum in the prepupa and then very rapidly falls to a low value in the early part of metamorphosis. These variations are in accordance with the results of Babers (*3*) and other investigators. Thus, whatever the causes of glycogen storage, the stored glycogen is utilized during metamorphosis. Since the blood-cell glycogen increases with increase of carbohydrate intake and decreases during starvation, indicating that conversion from and to glucose occurs, it seems more probable that the blood-cell glycogen is stored carbohydrate reserve than that it represents the transport of carbohydrate from the intestine during alimentation. Thus, one function of the blood cells appears to be to serve, like other tissue cells, for the storage of glycogen to be used during metamorphosis. Whether the glycogen is utilized by the blood cells themselves, as they play their roles during development, or whether their glycogen is made available as energy for other tissue cells is not evident from available data.

KINDS OF BLOOD CELLS CONTAINING GLYCOGEN

The results reported here show that when sufficient glucose was ingested by larvae most of the blood cells contained glycogen inclusions. There seemed to be one cell type, however, in which glycogen inclusions were not observed which, in the preparations stained by the Bauer technique and counterstained with thionin, appeared as an irregularly outlined cell with a relatively small nucleus that seemed to consist of a number of chromatin granules (pl. 2, *A*, *i*, and *B*, *f*). These cells are here called "rhagmatocytes" in accordance with a tentative cell classification. The oenocytoids also were not observed to contain glycogen.

The plasmatocytes usually showed a greater tendency to contain glycogen than did the cystocytes, although in a few preparations the cystocytes were observed to contain glycogen when little or none was detected in the plasmatocytes. Of the several authors who have reported the occurrence of blood-cell glycogen, Bogojavlensky (*14*) appears to be the only one who has attempted to identify the types of blood cells that contained the glycogen. In the walking stick *Carausius morosus*, and by implication in several other Orthoptera, Bogojavlensky found glycogen inclusions in spindle-shaped cells that were capable of becoming rounded, and that composed about 70 percent of the blood-cell population, but he did not state whether glycogen was found in the spindle-shaped cells, capable of becoming rounded, which comprised his second class of blood cells in *Bombyx mori* and by implication in several other Lepidoptera, and which would seem to correspond to the cells of *Prodenia eridania* here called plasmatocytes. Similarly, Bogojavlensky found glycogen in the proleucocytes of *C. morosus*, but not in those of *B. mori*. He also found a small amount of glycogen in his round cells with small nuclei, or micronucleocytes. He also reported that the granules of the granular leucocytes of *C. morosus* gave an iodophilic reaction with Lugol's solution and contained a carbohydrate (glycoproteid) component. Considering the fact that blood-cell glycogen is markedly influenced by carbohydrate absorption, the observations of Bogojavlensky and those reported here are not necessarily inconsistent, especially since there is not complete agreement with respect to insect blood-cell types and since *P. eridania* was used here and *B. mori* and other species were used by Bogojavlensky. The present observations and those of Bogojavlensky, that certain blood-cell types tend to contain more glycogen than other types, are in accordance with the observations (*4*, *21*, *29*, *42*) that the granular leucocytes but not the mononuclears of vertebrates contain glycogen.

FORMS OF GLYCOGEN INCLUSIONS IN BLOOD CELLS

It has been shown that glycogen inclusions may have different forms in different kinds of blood cells. Thus, the cystocytes characteristically contain relatively small granular inclusions. Certain other cells may seem to be more or less diffusely impregnated with glycogen and also to contain relatively large round or nearly round inclusions. In accordance with a tentative blood-cell classification, these cells are here called spheroidocytes. The plasmatocytes characteristically contain smooth-contoured, round, ovoid, or somewhat irregular glycogen inclusions, which may differ greatly in size, even within a single cell. Bogojavlensky (*14*) described similar glycogen inclusions in the spindle-shaped blood cells of *Carausius morosus*, and the glycogen inclusions figured by Vaney and Maignon (*104*) seem to be of analogous forms. The glycogen inclusions in the vertebrate granulocytes and pus cells described by some of the authors already cited seem to be small and of fairly uniform size.

The observations that some of the glycogen inclusions may be faintly stained or stained deeply at their peripheries but only faintly or not at all in their centers can be variously interpreted. They suggest that glycogen might occur either uniformly throughout the inclusion or be locally concentrated at the periphery. In no inclusion did glycogen appear much more concentrated at the center than at the periphery. The plasmatocytes frequently contained colorless vacuoles of the same general aspect as the glycogen inclusions and, like the glycogen inclusions, were often located at the poles of the fusiform cells. Bogojavlensky (*14*) found polar vacuoles in spindle-shaped cells of *Carau-*

sius morosus when they were observed in vivo, and from comparison of them with Best and Lugol stained preparations he concluded that they contained glycogen. It would thus appear either that chromic acid fixation followed by the Bauer staining procedure incompletely fixes and stains some of the glycogen in the blood-cell inclusions, or that blood-cell glycogen might be stored in preexistent cytoplasmic vacuoles or might be mobilized from and leave behind glycogen-free cytoplasmic vacuoles. The fact that, even within a single plasmatocyte (from a glucose-fed larva), deeply stained glycogen inclusions may vary from tiny granules to approximately nuclear size indicates that glycogen may also appear in the cytoplasm without being associated with large preexistent vacuoles. Sometimes the large colorless vacuoles and glycogen inclusions were found to occur at the same time in a single blood cell.

THE GLYCOGEN COUNT AND THE GLYCOGEN INDEX

With few exceptions the individual glycogen counts were of the same general form as the average counts shown in figure 1, *B* and *C*. The exceptional individual counts were similar in form to that represented by circles in figure 1, *E*, in that the number of cells in class 3 exceeded the number in class 3+. Examination of the smears corresponding to the exceptional counts showed that few or none of the cystocytes contained glycogen inclusions. Figure 1, *E*, also shows that class 3+ may exceed class 3 in number of cells because the cystocytes tend to enter class 3+ more readily than classes 1, 2, and 3. Figure 1, *D*, shows that as the glycogen index increases the number of cells in classes 1, 2, 3, and 3+ increases, but that the cells of class 1 increase relatively more than those of class 2, which increase relatively more than those of class 3.

It appears, therefore, that as the glycogen index increases two shifting processes occur in the glycogen count. First, cells may pass relatively slowly from class 0 through classes 1, 2, and 3 to class 3+. Such a process would tend to cause a shift of the maximum from class 0 to class 1, when the glycogen index becomes sufficiently high. This is shown by figure 1, *D*, to have actually occurred. Theoretically, the maximum should shift further from class 1 through classes 2 and 3 to class 3+, provided no limiting factors hinder the processes of glycogen accumulation in the blood cells as the glycogen index becomes still higher. It is probable, however, that limiting factors do exist in the insect and would prevent the maximum from shifting all the way to class 3+. Secondly, cells may pass very rapidly from class 0 through classes 1, 2, and 3 to 3+, or they may move from class 0 to 3+ without going through the other classes at all. Either process would cause a greater number of such cells to remain in class 3+ than in classes 1, 2, and 3. Figure 1, *A–E*, shows that actually class 3+ tends to be greater than class 3 in numbers of cells, and figure 1, *E*, indicates that, with regard to cystocytes, class 3+ exceeds classes 1 and 2 as well as class 3.

The microscopic observations also are in accordance with this interpretation of these figures. The cystocytes tend to contain glycogen inclusions in the form of a relatively large number of small granules, whereas the plasmatocytes tend to contain fewer glycogen inclusions of relatively large size (pl. 2, *C*; 5, *C* and *D*).

These results indicate that, in the glycogen count, as the index increases the plasmatocytes shift according to the first (slow) and the cystocytes according to the second (rapid) process.

STATE OF THE BLOOD-CELL GLYCOGEN

The blood-cell glycogen reported here includes only that which is histochemically detectable, whereas that reported by Babers (*3*) includes that which is determined by chemical analysis. It should not be assumed that the histochemically and chemically determined forms of glycogen are identical (*4*, *76*). Bakker (*4*) found that chemically and histochemically detectable glycogen of exudate leucocytes may differ. Willstätter and Rohdewald (*113*) indicated that the glycogen of horse leucocytes exists mostly as a soluble lyoglycogen and to some extent as a less soluble, protein-bound desmoglycogen which, unlike the lyoglycogen, does not show the iodine staining reaction. They (*112*) also indicated that, when the leucocytes leave the circulating blood and migrate through the tissues, some conversion of desmoglycogen into lyoglycogen occurs. Bierry, Gouzon, and Magnan (*13*) reported that hepatic glycogen occurs as free (soluble) and protein-bound (insoluble) glycogen. Other evidence (*86*, *87*, *88*) also indicates the possibility of glycogen-protein combinations occurring in cells. Wajzer (*107*) found that, in the frog and the guinea pig, a large proportion of the liver glycogen normally occurs in combination with protein (proteid glycogen). Bogojavlensky (*15*) found that fat-body glycogen of the silkworm is much less soluble than the glycogen in other tissues. It is therefore quite possible that insect blood cells may contain lyo- and desmo- forms of glycogen, but distinction between them will depend upon future investigation.

GLYCOGEN IN ALIMENTARY EPITHELIAL CELLS

The results reported here indicate that, in the normal course of development of *Prodenia eridania*, glycogen is absent from the midgut epithelial cells and the blood cells at the time of hatching, that it appears earlier in the gut cells than in the blood cells, that it occurs in gut cells in each instar, and that individual variations are large. This is in accordance with the results of Babers (*3*), who found no glycogen in the larva at the time of hatching. It cannot be concluded from these observations that the glycogen of the midgut epithelial cells is synthesized from ingested glucose as a part of the absorption process, for it is possible that these cells, like those of the blood and the other tissues, are able to store up carbohydrate reserves from glucose in the blood. The question of the occurrence of glycogen synthesis in the intestinal epithelia of vertebrates as a part of the mechanism of glucose absorption is as yet unsettled; in fact, it seems that it does not occur (*106*, *53*, *68*). Nevertheless, glycogen has been reported in the alimentary epithelium of the mammal by Rouget (*94*), Creighton (*22*), Von Fürth (*37*), and Horne and Magee (*53*); of the frog by Barfurth (*5*); of the fly larva (epithelium?) by Bernard (*8*); of the silkworm by Bogojavlensky (*15*) and Paillot (*78*, *79*); of *Limax* by Barfurth (*5*); of *Daphnia* by Von Dehn (*24*) and Beutler (Gallistel *39*); and of *Chironomus* larva by Harnisch

(*46*). Von Dehn (*24*) found gut-cell glycogen variable in *Daphnia* and, in general, detected it histochemically in animals that had been fed starch but not in starved animals. Gallistel (*39*) reported glycogen in the midgut cells of *Daphnia* only when there was an abundance of fat and glycogen in the fat-body cells. Bogojavlensky (*15*), making use of starvation and subsequent feeding procedures, reported that, after prolonged starvation of silkworms, glycogen remained only in the wall of the anterior part of the hindgut, the other tissues containing none, and that, with subsequent feeding, glycogen occurred first in the fat-body cells and later in the midgut epithelium. Vaney and Maignon (*104*) failed to find glycogen in the digestive epithelium of the silkworm, and De Boissezon (*16*) did not detect it in the epithelia of the midgut and hindgut of *Culex pipiens*.

From the information thus available it would seem that glycogen occurs generally, but variably, in the gut epithelia of animals, that the gut-cell glycogen varies with the intake of carbohydrate, and that it probably represents a mere storage of carbohydrate reserve rather than a glycogen synthesis that acts as a part of the glucose-absorption mechanism.

GLYCOGEN IN OTHER TISSUES

The histochemical detection of glycogen in other tissues of insects has been reported as follows:

Tissue and insect	*Investigator*
Fat body:	
Fly larva	Bernard (*9*).
Silkworm	Vaney and Maignon (*104*), Bogojavlensky (*15*), and Paillot (78, 79).
Tent caterpillar	Rudolfs (*96*).
Mosquito	De Boissezon (*16*).
Chironomus larva	Harnisch (*46*).
Oenocytes:	
Lepidoptera	Hollande (*51*).
Aquatic Hemiptera	Poisson (*85*).
Silkworm	Bogojavlensky (*15*) and Paillot (79).
Pericardial cells:	
Gryllus campestris and *Carabus auratus* L. (after injection of glucose)	Hollande (*52*).
Silkworm	Paillot (*79*).
Malpighian tube:	
Silkworm	Paillot (*79*).
Chironomus larva	Harnisch (*46*).
Reproductive system, silkworm	Bogojavlensky (*15*) and Paillot (*79*).
Eggs, etc	*See* literature cited by Babers (*3*).
Nervous system (ganglia), silkworm	Bogojavlensky (*15*).
Hypodermal cells, silkworm	Bogojavlensky (*15*) and Paillot (78, *79*).
Muscle:	
Silkworm	Vaney and Maignon (*104*).
Tent caterpillar	Rudolfs (*96*).
Mosquito	De Boissezon (*16*).
Silkworm	Paillot (*79*).
Chironomus larva	Harnisch (*46*).
Associated with tracheal walls, silkworm	Bogojavlensky (*15*) and Paillot (*78, 79*).

In the present investigation no polysaccharide was observed in the tracheal walls of *Prodenia eridania.* The ventral ganglia contained polysaccharide, and Bogojavlensky (*15*) made similar observations in the silkworm. The polysaccharide observed in the armyworm ganglia might or might not be located in the nervous tissue itself. Havet (*47*) reported that glycogen in the nerve centers of several animals, including *Limax*, is contained only in the neuroglial cells. Schabadasch (*97*) reported glycogen as occurring in both glial and neural cells in the mammal. Vaney and Maignon (*104*), Bogojavlensky (*15*), and Paillot (*79*) all found that striated muscles in the silkworm appeared to contain either no glycogen or a small amount spread diffusely throughout the substance of the fiber; but none of these authors fed large quantities of glucose to the insects. When *Prodenia eridania* larvae were fed large amounts of glucose, their striated muscles were found to be heavily loaded with polysaccharide, which appeared to be diffuse throughout the fiber but relatively concentrated at the cross striations; the alary muscle fibers, however, were relatively poor in polysaccharide. That histochemically detectable polysaccharide is definitely related to the cross striations of muscle in vertebrates is indicated in papers by Arnold (*1*), Creighton (*22*), Von Studnitz (*101*), Noll and Becker (*77*), and Feyel (*32*). Glycogen was also observed to occur in the labial glands of *Prodenia eridania*, but Vaney and Maignon (*104*) and Paillot (*79*) failed to detect it in these glands of the silkworm.

Since ingested glucose influences the polysaccharide content of tissues, disagreements of reported observations may be due in part to possible differences in food intake by the insects observed. These observations on *Prodenia eridania* indicate that many of the larva's tissues have the capacity to store up much more polysaccharide than they seem ordinarily called upon to store.

Although glycogen has been reported in the nuclei of some animal cells (*17*, *62*), polysaccharide has not been observed in the nuclei of any of the blood or other tissue cells of *Prodenia eridania*, insofar as the nuclear structures in these tissue smears could be distinguished.

SITE OF GLYCOGEN SYNTHESIS

Bogojavlensky (*15*) reported that the fat body of the silkworm is the site of the first glycogen synthesis during poststarvation food intake. Babers (*3*) showed that, whereas the tissues of the normal sixth-instar *Prodenia eridania*, exclusive of the blood and alimentary tract, contained on an average about 4 percent of glycogen, dry weight, the isolated fat-body tissue contained about 23 percent. Although it is possible that glycogen synthesis may occur solely or chiefly in the fat body, whence it could be transported to other tissues, this does not seen probable, for Babers (*3*) found practically all the blood glycogen of this insect localized in the blood cells and almost none in the plasma. It is more likely, therefore, that the blood cells themselves possess a mechanism for gluconeogenesis and glycogenolysis.

INFLUENCE OF DIFFERENT HISTOCHEMICAL PROCEDURES

Some difference of opinion has been expressed with regard to the adequacy of different fixatives used in the histochemical detection of glycogen (*67*, *pp. 226–227*). Various procedures are recommended for use with insects. Brammertz (*17*) used Carnoy's fluid and Best's carmine stain (controlled by the iodine and salivary tests) to demonstrate glycogen in the embryos of *Apis mellifera* L. (*A. mellifica*),[7] *Bombyx mori*, and *Meloe proscarabaeus* L. (*M. carabaeus*). Bogojavlensky (*15*) fixed silkworm tissues in Carnoy's fluid and in saturated solution of corrosive sublimate, saturated with dextrose, and reported Carnoy's fluid satisfactory for glycogen fixation. De Boissezon (*16*) used absolute alcohol and Best's carmine to fix glycogen in *Culex pipiens* L. Gendre (*40*) modified Bouin's fixative by replacing the water with 95-percent alcohol saturated with picric acid, and reported the modified fluid satisfactory for glycogen fixation in silkworm tissues. Paillot reported Gendre's fixative as satisfactory for glycogen fixation in the fat body (*78*) and in the hypodermal cells (*79*) of the silkworm.

For glycogen detection in tissues of animals other than insects, Carnoy's fluid, absolute alcohol, and the fixatives of Pasteels and Léonard and of Gendre are recommended. Von Studnitz (*101*) used absolute alcohol and Lugol's solution to demonstrate glycogen in the frog's sartorius muscle. Noll and Becker (*77*) used absolute alcohol and Best's carmine to demonstrate glycogen in muscles of the rabbit and the hen. Gallistel (*39*), studying glycogen in *Daphnia magna* Straus, fixed glycogen chiefly with Carnoy's fluid but also with absolute alcohol and stained by Best's carmine method or the iodine method of Langhans. Havet (*47*), in a study of glycogen in the nervous systems of the rat, the cat, and *Limax*, fixed with absolute alcohol or 92-percent alcohol containing iodine, and stained by Best's method or by the iodine method of Prenant; he considered formol and Bouin's fixative unsatisfactory. Gendre (*40*), using his own fixative, reported satisfactory glycogen fixation in the dog, adult monkey, fetus of monkey, adult rat, newborn rat, and silkworms; he stained by methods of Lugol, Best, and Bauer. Coutelen and Cochet (*20*), in a study of glycogen in the larvae of cestodes, found absolute alcohol better than water fixatives, with or without picric acid, better than formol saturated with glucose or corrosive sublimate saturated with glucose, and better than the fixatives of Bouin, Zenker, Carnoy, Flemming, and Altman; Coutelen and Cochet used their own iodine staining technique. Feyel (*32*), in a study of glycogen in striated muscle fibers and liver of the frog, employed fixation in absolute alcohol with paraffin embedding, and also Bouin-Allen, Gendre, and Pasteels-Léonard's fixatives, each with dioxane-paraffin or celloidin embedding; she stained by the gum-iodine method, the iodine method of Langhans, Best's carmine method, and Bauer's method, and employed the salivary counterproof method. Feyel concluded that the best results were obtained by fixing the muscles for 20 to 24 hours in Bouin-Allen fixative and embedding in celloidin. Pasteels and Léonard (*81*) studied the demonstration of glycogen in embryonic tissue and in adult liver by fixing the tissues in absolute alcohol for 24 hours, in alcoholic formol for 24 hours, in formol saturated with dextrose for 24 hours, in Carnoy's fluid for 4 hours, in Zenker's fluid for 24 hours, in the Bouin-Allen fixative for 6 hours, and in their own fixative; they stained by the methods of Lugol, Best, and Bauer, and employed the salivary counterproof. They concluded that the most satisfactory fixative for glycogen was an aqueous fixative saturated with picric acid, that the Bouin-Allen fixative was satisfactory for hepatic glycogen, that aqueous fixations were required for blastulae, gastrulae, etc., and that their own dioxane-picric acid-formalin-acetic acid liquid was satisfactory for fixation of glycogen.

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[7] Scientific names of insects in parentheses immediately following other scientific names are those appearing in the reference cited. Other scientific names are those currently in use according to the Division of Insect Identification of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

Lison (*67, p. 230*) stated that the Best carmine method has no histochemical specificity. Lee (*64, p. 288*) recommended that preparations be made in tripilicate, to be used with the iodine method, Best's carmine method, and the salivary counterproof method. Bauer (*7*) reported that his method would selectively demonstrate polysaccharides such as glycogen, galactogen, starch, tunicin, and cellulose. Lison (*67, p. 232*) considered that, inasmuch as the coexistence of many polysaccharides in a given biological location is unlikely, Bauer's method is a good histochemical test for glycogen. Lee (*64, p. 289*) considered Bauer's method to be less specific than others. Giovannola (*41*) concluded that the polysaccharide reaction of Bauer is better than the Best stain for many kinds of protozoa. Gendre (*40*) considered that the iodine reaction, with salivary counterproof, is specific, that the Best method is less specific than the Bauer method, which itself is not very specific; he recommended that the Bauer method be controlled by the mucicarmine method of Mayer and the method of salivary counterproof. Coutelen and Cochet (*20*) stated that, among the glycogen methods they used, only the iodine method and the Bauer method possess histochemical value and that, as the latter is positive for other polysaccharides than glycogen, it should be accompanied by other methods. Bakker (*4*) obtained results indicating that positive and negative iodine reactions are insufficient evidence upon which to decide whether or not a cell contains glycogen.

In the present work the fluids of Gendre, Bouin, Bouin-Allen, Pasteels and Léonard, Neukirch, and Vastarini-Cresi gave unsatisfactory glycogen fixation when applied as indicated in table 1. These fluids were applied to blood and tissues of larvae that had been heat-fixed, and the blood and tissue smears were not subjected to the usual dehydrating and embedding procedures. None of the fixatives—absolute alcohol, Carnoy's fluid, and Zenker's fluid—that were found to give better results (table 1) contained picric acid.

The staining procedures used here were chiefly the method of Bauer, which was supplemented with Lugol's iodine method, the method of Best, and as counterproofs, Mayer's mucicarmine method and the method of salivary digestion, insofar as blood and midgut epithelial cells were concerned. The results are considered to indicate that the polysaccharide in the blood and the gut cells is glycogen, but since only the Bauer method was used on most of the other tissues, it cannot be concluded that the polysaccharide occurring in those tissues is glycogen. The results of this investigation, however, taken in conjunction with those reported by the other investigators, indicate that the observed tissue polysaccharide is probably glycogen.

SUMMARY AND CONCLUSIONS

Observations on the occurrence of glycogen in blood cells and midgut epithelial cells, as well as of polysaccharide in various other tissues, of the southern armyworm (*Prodenia eridania* (Cram.)) are presented and discussed with reference to other literature bearing upon the subject. The results of several experimental procedures are also presented. From the observations and results reported here, the following conclusions may be drawn in regard to histochemically detectable glycogen or polysaccharide in the southern armyworm:

(1) Glycogen occurs normally in the blood cells, midgut epithelial cells, and certain other tissue cells of the larvae.

(2) Neither blood-cell glycogen nor midgut-epithelial-cell glycogen occurs normally in the larva at the time of hatching.

(3) As indicated by values of the glycogen index, blood-cell glycogen increases during normal larval development until it attains a maximum in the prepupa; after pupation it rapidly decreases and remains at a low level during most of the pupal period, tending to disappear toward the end of the pupal period. In the first instar, and probably in the adult, blood-cell glycogen occurs very infrequently.

(4) When newly hatched larvae are kept without food, neither blood-cell nor midgut-epithelial-cell glycogen occurs up to the time of death; but when they are given food containing sufficient carbohydrate, particularly glucose, midgut-epithelial-cell glycogen appears. It tends to appear sooner than blood-cell glycogen, which occurs infrequently.

(5) In sixth instars blood-cell glycogen decreases or disappears during starvation and increases after ingestion of food containing carbohydrate, particularly glucose.

(6) As many as 85 percent of the larva's blood cells may contain glycogen inclusions as a result of ingestion of sufficient carbohydrate, particularly glucose.

(7) Most, but apparently not all, of the insect's blood-cell types can contain glycogen inclusions as a result of glucose ingestion.

(8) The blood cells here called plasmatocytes and cystocytes showed a greater tendency to contain glycogen inclusions than did the other blood-cell types. When the glycogen index is sufficiently high, glycogen inclusions may appear, however, in the cells here called spheroidocytes, polypodocytes, and nematocytes, as well as in some others. Glycogen inclusions may also occur in cells undergoing mitotic division.

(9) The glycogen inclusions tend to be fewer and larger in the plasmatocytes than in the cystocytes.

(10) The glycogen count of the normal insect typically has the form $0>1>2>3<3+$, where the numbers refer to the classes in the count.

(11) As the glycogen index increases, the cells decrease in class 0 and increase in the other classes. The increase is greater in 1 than in 2 and greater in 2 and 3+ than in 3. When the glycogen index increases enough, the maximum shifts from class 0 to class 1.

(12) The plasmatocytes tend to pass through all the classes from 0 to 3+ relatively slowly, whereas the cystocytes tend either to pass through them rapidly or to enter class 3+ without passing through 1, 2, or 3 at all.

(13) Average glycogen counts from nonligatured larvae and from the fore and hind ends of ligatured larvae have similar forms.

(14) Glycogen may occur in midgut epithelial cells in each instar.

(15) Midgut-epithelial-cell glycogen is influenced by food ingestion, decreasing as a result of starvation and increasing after high intake of carbohydrate, especially glucose.

(16) Glycogen inclusions can occur in the labial-gland cells and the oenocytes of first instars.

(17) Polysaccharide can also occur in the following tissues of larvae fed sufficiently large amounts of glucose-cornstarch-turnip leaf food: Ganglia (and connectives) of the ventral nerve cord, labial glands, Malpighian tubes, fat body, oenocytes, gonads, pericardial cells, integument, walls of the foregut and hindgut, striated muscle fibers of body muscles, gut musculature, cardiac muscle, and the alary muscle fibers.

(18) In the striated muscle fibers the polysaccharide occurs diffusely in the fibers and concentrated at the cross striations.

The observations and results reported here, taken in conjunction with the other results reported in the literature cited in this paper, tend to support the following conclusions:

(1) *Prodenia eridania* is not exceptional in that some of its circulating blood cells normally contain glycogen inclusions.

(2) The blood-cell glycogen represents a store of reserve carbohydrate rather than, for example, a newly absorbed foodstuff that is being transported by the blood to the tissues; hence, storage of reserve carbohydrate may be recognized as one function of the southern armyworm's blood cells, particularly the plasmatocytes and the cystocytes.

(3) The glycogen-containing blood cells themselves possess mechanisms for gluconeogenesis and glycogenolysis.

(4) Gluconeogenesis and glycogenolysis may be influenced by a number of different factors, which are discussed.

(5) The midgut-cell glycogen indicates a storage of reserve carbohydrate rather than a glucose-absorbing mechanism.

(6) Glycogen synthesis occurs in those tissues in which the glycogen appears, rather than exclusively in a single particular tissue, such as the fat body, although this body may synthesize and store a relatively large amount of glycogen.

(7) Glycogen probably occurs in the glycogen-storing cells in both a soluble and a relatively insoluble form.

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"PARTICLES" AND THEIR RELATIONSHIP TO THE STRUCTURE OF ANIMAL FIBERS [1]

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INTRODUCTION

Many of the problems concerned with the variation in the qualities of animal fibers can never be solved until a more comprehensive understanding of the minute structure of the fibers has been achieved. Of the many animal fibers, wool is the most widely used. The peculiar properties of wool have led to much speculation as to the structure of the fibers. More than a century ago Thomas Plint, a woolen manufacturer, made the following statement, as quoted by Youatt (*24, p. 84*):[2]

> Respecting the application of the microscope to the examination of the fibre, I am decidedly of opinion that a careful and minute examination of wools differing in their felting properties would issue in the detection of some specific difference of structure. This property is altogether inexplicable, at least in my mind, except on the supposition that the extreme surface of the fibre is irregularly *feathered*, and that, by compression, these feathered edges become entangled and locked together. These feathers must also point in one direction, viz, from the root to the extremity of the fibre; and if we suppose the feathered edge, or, more properly speaking, the individual tooth or feather, to be of a firm texture, it is evident that one tooth being pushed into another, would fasten like a wedge; and if we further suppose that the tooth or feather has a barb, similar to that on a whale-harpoon, the phenomena of felting are explained.

Plint's observation of the serrated edges of wool is also described by Youatt (*24, p. 87*), as follows:

> A fibre was taken from a Merino fleece of three years' growth * * *. It was taken without selection, and placed on the frame to be examined as a transparent object. A power of 300 (linear) was used, and the lamp was of the common flat-wicked kind. The focus was readily found; there was no trouble in the adjustment of the microscope, and, after Mr. Powell [the maker of the microscope], Mr. Plint had the first perfect ocular demonstration of the irregularities in the surface of wool—the palpable proof of the cause of the most valuable of its properties its disposition to felt.

During the last century the larger elements of the structure of wool fibers were definitely established. The three main regions are the cuticle, cortex, and medulla. The cuticle is the layer of thin, horny, irregularly shaped, platelike cells that envelop the fiber. These cells may overlap each other or surround the fiber, but their edges always point toward its tip. Many patterns are formed on the surface of wool fibers by the arrangement of these cells. They differ in different animals; in fact, they vary from the root to the tip end on many fibers. The cortex, which lies just below the cuticle, constitutes most of the remaining portion of a wool fiber and sometimes all of it. It is made up of spindle-shaped cells that are intimately

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[2] Italic numbers in parentheses refer to Literature Cited, p. 302.

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bound together, probably by some sort of binding substance. The strength and elasticity of wool are attributed largely to these horny cells. Many fine fibers are composed of only the cuticle and the cortex; however, in most wool fibers there is a central corelike structure, the medulla. It may differ in appearance in different kinds of wool.

Nathusius (*14*) disintegrated wool fibers by chemical means, employing sulfuric acid, alkali, or ammonia to separate the cells. He found the cuticle, cortex, and medulla to be composed of cells of different shapes. McMurtrie (*10*), employing the same reagents, examined these cells in detail with special reference to differences among various breeds of sheep. Walther's (*22*) ambitious measurements of 20,000 cortical cells, separated by sulfuric acid, showed that there are slight differences in size of wool-fiber cells among different breeds of sheep but they are not of sufficient magnitude to warrant definite conclusions.

Since disintegration of the fibers by chemicals always produces some swelling, attention was turned to biological methods of disintegrating fibers. Meunier, Chambard, and Comte (*11*) successfully employed an enzyme, pancreatin, for the separation of the cortical cells, and also of the cuticular cells. Waters (*23*) isolated the bacterium that causes pink rot of wool, and this organism was used by Gabriel (*6*) to break up the fibers for the study of the cellular characters of wool from Merino, Romney, and Lincoln sheep. Burgess (*2*) disintegrated wool fibers by the action of the enzymes trypsin or pepsin. The results were similar to those obtained with the proteolytic bacteria but were achieved much more rapidly.

Although the early workers thought that the cell represented the ultimate element of structure of animal fibers, later studies have disclosed smaller features. Striations lengthwise of the fibers have been noticed for a long time. Waldeyer (*21*), in examining hairs, concluded that these were fibrils ("Hornfibrillen"), which were constituent parts of cells, but were longer than individual cells. He believed them to be the elements of the structure of fibers, and he thought that they were produced by the cell protoplasm. Nathusius (*15*) confirmed the finding of these fibrils and their importance as structural units of the cortical cells but did not find substantiation of Waldeyer's (*21*) claims that the fibrils were larger than entire cells. Moreover, he also mentions an interfibrillar cementing substance.

Physicists and chemists are at present approaching the study of fiber structure from entirely different angles. Thus Astbury (*1*) is determining the shapes and the dimensions of the molecules and the way they fit together. Speakman (*19*), Harris and Rutherford (*8*), and others are explaining characteristic properties of wool in terms of chemical differences.

In the opinion of the writers, there is a large gap between the fields in which work has been in progress on wool. On the one hand, the microscopists examine wool at 300 to 500 diameters' magnification, usually to understand its gross structure, whereas the physicists and chemists study the size, shape, linkages, and other characteristics of the structural components of fibers in terms of angstroms (0.0001μ). However, exact knowledge of the smallest visible units of structure of the fibers and the manner in which they are held together is yet very meager. Indications of the existence of particles in wool, mohair, human hair, cattle hair, and hog bristles were obtained by Hardy and reported by Mohler (*12, p. 26*). Questions arise as to what may be revealed concerning the structure of the cuticle, the cortex, and the medulla when they are examined at higher magnifications than have ordinarily been used, and also whether details can be more sharply defined by special attention to optical and histological techniques at all magnifications. To obtain information on these points, the study reported in this paper was conducted from 1938 to 1940 at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md.

EXPERIMENTAL METHODS

In seeking units of structure of animal fibers smaller than the fibrils reported by Waldeyer (*21*), wool fibers from Lincoln and Karakul sheep were chosen as the material for investigation. Several methods were employed to disintegrate the fibers in order to avoid artifacts. Mechanical disintegration was accomplished in two ways: (1) The fibers were dissected under the microscope (Greenough type), bits of the fiber being peeled off the surface with a sharp knife, and mounts made of these surface or cuticular scrapings and also of scrapings of the cortex; (2) fragments of fibers in a more finely divided state were obtained by grinding the sample in a Wiley mill and removing the coarser fragments by sifting the ground material through a fine-mesh glass cloth.

Chemical disintegration of the cells was obtained by a procedure similar to that followed by earlier investigators. This consisted in immersing mounted fibers in a warm solution of equal parts of sulfuric acid and water or 5-percent sodium hydroxide. Two or three fibers were mounted on a glass slide with adhesive tape, the fibers extending nearly the entire length of the slide. As the fibers disintegrated, successive segments were removed from the slide and mounted in glycerin. Slight pressure was applied to separate the fiber cells. In this manner slides were obtained that showed the various stages in the separation of the cells. Saturated aqueous solution of bromine, as well as perhydrol, was found to be satisfactory for disintegrating the fibers.

Finally, a new biological method of disintegrating the fibers was devised by the senior author, as reported by Mohler (*12*). It consists in feeding wool to carpet beetles,[3] *Anthrenus vorax* and *Attagenus piceus*, under controlled conditions. The discovery of this method resulted from an inspection of guinea-pig-hair samples that had been stored in large gelatin capsules. The snow-white hair had somehow been replaced by a snow-white powder; black and white hair by a gray powder. Closer inspection revealed a small hole in each capsule; the larvae of some insect had eaten through the gelatin of the capsules, devoured the hairs, and made their exit the same way that they had come. The entire sample cabinet proved to be infested with carpet beetles. It seemed logical to assume that if the beetles had lived only on the hair, their excreta would contain remains of fibers in a very finely divided state. Microscopical examination confirmed this. This lead was followed up by an experiment. Carpet beetle larvae were placed in contact with various kinds of animal fibers, including several kinds of wool, hog bristles, and the extremely tough bristles from the tail of the African elephant. All of these were consumed with apparently equal ease and relish by the carpet beetle larvae. Passing through the digestive tracts of the insects, these animal fibers were reduced to a powder.

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[3] These were obtained through the courtesy of Ernest A. Back, of the Bureau of Entomology and Plant Quarantine, and of William A. Davidson, formerly of the Food and Drug Administration, U. S. Department of Agriculture.

In view of this finding, the following experiment was performed: Fifty of the beetle larvae were put into each of a series of specimen jars having a capacity of about 25 cc. and provided with screw caps. A sample of wool was added to each jar, which was then set in a dark place at room temperature. In a few days the wool was reduced to powder by passing through the digestive tracts of the beetles.

To determine the arrangement of the particles in the fibers, cross sections and longitudinal sections were cut on a rotary microtome.[4] The fibers were prepared for sectioning by putting them through three changes of dioxane and then keeping them in a paraffin oven for 2½ hours in a mixture of one-third dioxane and two-thirds soft paraffin. At the end of that time they were transferred to hard paraffin (56° 58° C.), left for 2½ hours, and then embedded. The best success in cutting the sections was found with blocks that had stood about 1 week before cross sectioning.

Mounted specimens derived by the above methods were examined under different microscopic conditions, including ordinary transmitted light, oblique illumination, and polarized light. It was not practical to measure directly the small individual units found. However, by the use of an ocular step micrometer it was possible to observe chains of the minute structural units of the fibrils and to measure the length of several chains, from which the approximate size of individual units could be determined.

EXPERIMENTAL RESULTS

Both the scrapings of the fibers and the fibers that had been ground and passed through the fine-mesh glass cloth were found to be too

EXPLANATORY LEGEND FOR PLATE 1

A, Aggregates of particles with cementing material in a finely ground Lincoln fiber; mounted in acryloid.

B, Aggregates of particles in a finely ground Lincoln fiber; mounted in polystyrene.

C, Individual particles in finely ground Karakul fibers; mounted in polystyrene.

D, Individual particles in excreta of carpet beetles fed Lincoln fibers; mounted in polystyrene.

E, Striated appearance of an untreated Karakul fiber, as revealed by an impression on a transparent thermoplastic film.

F–I, Progressive stages in the disintegration of fibers through the use of sodium hydroxide: *F*, fiber swollen, chains of black particles visible in lighter edges of Karakul fiber; *C*, Karakul fiber greatly swollen, particles evident in medulla (corelike center); *H*, partial disintegration of Karakul fiber, medulla still preserved, cells broken down into fibrils: *I*, complete disintegration of Lincoln fiber, fibrils separated.

J, Cross section, about 5µ thick, of Karakul fiber, showing aggregates of particles.

K and *L*, Longitudinal sections of Karakul fibers, showing regular alignment of chains of particles. (*A–D*, × 3600; *E–L*, × 600)

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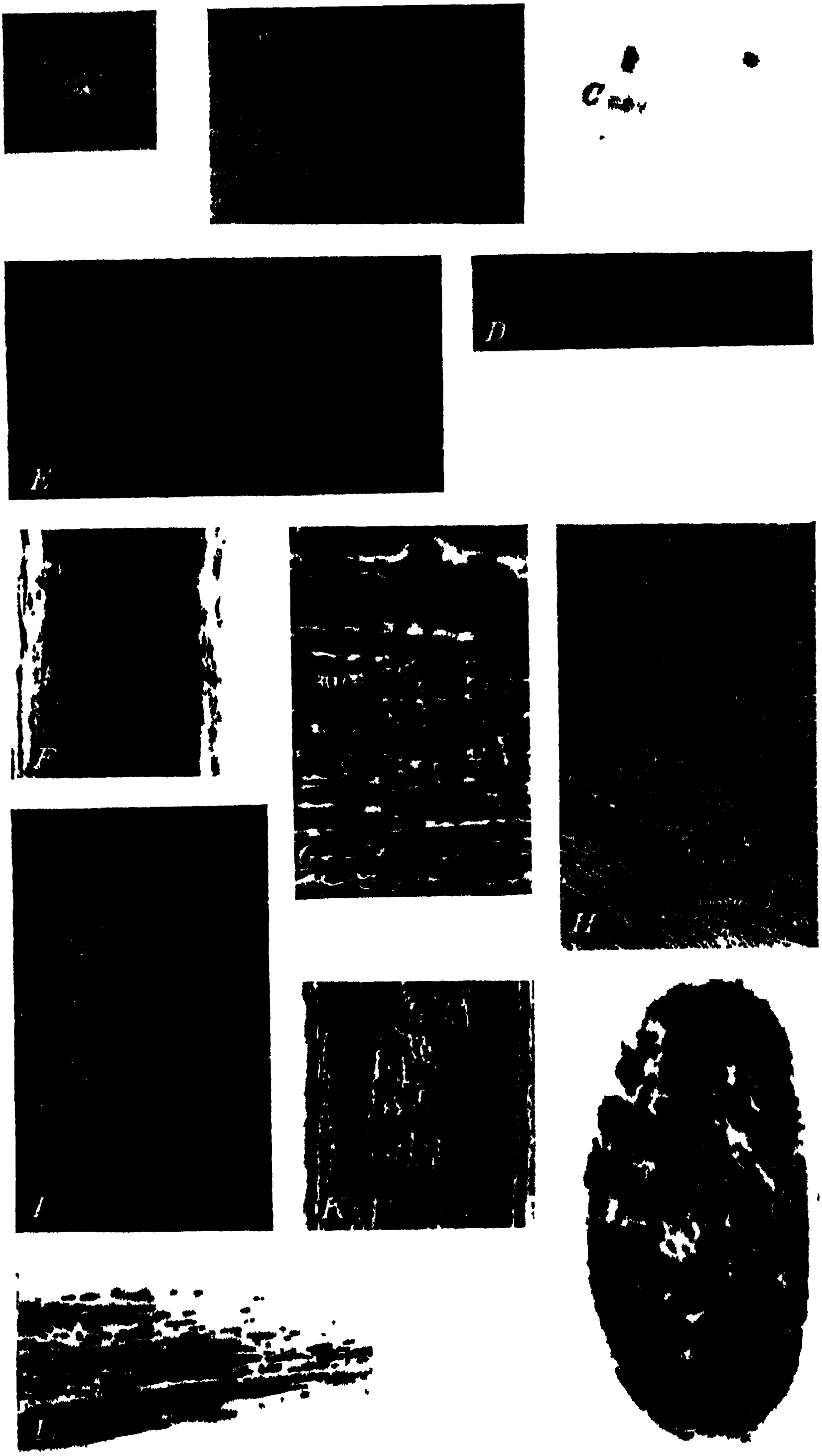
[4] These sections were prepared by H. Dean Ray, of the Bureau of Animal Industry.

PLATE 1



large to show the details clearly. However, the fine dust sifted from the ground fibers was satisfactory. On examination of this powder under the microscope with oblique illumination, aggregates (pl. 1, *A* and *B*) and individual small spherical units (*C*) could be seen. These individual units, for which the authors propose the name "particles," were only a fraction of a micron in diameter.

The arrangement of the cells, fibrils, and particles was best observed in the specimens that had been prepared by chemical treatment. Plate 1, *E*, shows an impression of an untreated Karakul fiber on a transparent thermoplastic film, obtained by a method previously developed by the authors (*7*). The somewhat striated appearance is evidently caused by fibrils, which in turn are composed of chains of particles. *F*, *G*, *H*, and *I* show progressive stages in the chemical disintegration of fibers. In *F*, these chains of black particles are visible in the lighter edges of the fiber. In *G*, which shows a greatly swollen fiber, the particles may be clearly seen in the medulla. In *H*, the fiber is partly disintegrated although the medulla is still preserved. The cortical cells have broken down into fibrils, in which the chains of particles hold together in a colloidal mass. In *I*, the fibrils have become separated during severe swelling. Here, again, the particles are seen to be arranged in single rows of chains. In the cortex the cells appear in parallel chains, whereas in the medulla the chains are irregular and very much twisted. It is possible that these chains of particles are not in the same plane for their entire length and may, therefore, be longer than they appear. The arrangement of these particles in single rows of chains and the approximately parallel alignment of many of the fibrils with respect to the length of the fiber, may be of fundamental significance in determining some of the important mechanical properties of animal fibers.

Inspection of the fine powder to which the wool was reduced by passing through the digestive tract of the carpet beetles revealed that it was made up of aggregates of many small pellets. On crushing, these pellets were found to be composed of small spherical units that appeared to be the same as those obtained by the other methods.

Examination of parts of fibers from Karakul sheep by ordinary transmitted light greatly facilitated the recognition of the particles because of the presence of pigment in many of them (pl. 1, *J*, *K*, *L*). The significant fact is thereby established that the individual particles may contain pigment, as shown also in *C*. The pigmented particles are usually black but some brown ones have been seen. It is not uncommon for black particles and white particles to exist in close proximity in the same fiber, but in any one fibril particles of one color appear to predominate (*K*).

In plate 1, *J*, which shows a thin cross section of a Karakul fiber, particles may be seen in aggregates of different shapes and sizes. In *K* and *L*, which show longitudinal sections of Karakul fibers, chains of particles are aligned in a regular manner.

Under polarized light this alignment is further manifested. A whole chain exhibits the same characteristics of double refraction as do the individual particles. The fibrils are bright when placed in a position 45° to the planes of vibration of the Nicol prisms but are extinct when placed parallel to the planes of vibration. When the "first order red" plate is inserted, the chains are alternately blue and yellow as they are rotated through the quadrants. Since the particles are spherical, such behavior of the fibrils indicates that the particles are arranged in accordance with the alignment of submicroscopic internal structures.

Measurements of chains of particles yielded an estimated diameter of 0.6μ for individual particles. Particles obtained from finely ground Karakul fiber are shown in plate 1, *C*, and particles from the excreta of carpet beetles fed Lincoln wool fibers are shown in figure *D*, both mounted in polystyrene. A transparent film was observed repeatedly. Such films may be seen in pieces of finely ground Lincoln wool fiber mounted in acryloid (*A*). The particles are apparently attached to this film, which may therefore prove to be of the nature of a cementing material.

DISCUSSION

The particles are apparently composed of keratin. The question of the relation of these keratin particles to micelles and crystallites naturally arises. For many years scientists have attempted to gain an understanding of the smallest structural units. What they have seen and their interpretations have given rise to controversies.

The definition of micelles has undergone several revisions through the years. Nägeli's conception, as reported by Nägeli and Schwendener (*13*), was that molecules are grouped into submicroscopical, that is, invisible, crystals which he termed "micelles." Speakman still refers to micelles in wool 200 angstroms thick and probably 10 times as long. Frey-Wyssling (*5*) modified the concept of micelles to apply to all the long molecular chains which are the framework that lends rigidity to a gel. This is an extension of thought in that it includes not only the aggregates of chain molecules in sufficient numbers to produce crystalline effects, but also those composed of but few parallel chain molecules. On this basis he proposed a theoretical structure of fibers which he believed would reconcile the two conflicting points of view: on the one hand, the existence of a bricklike structure composed of a disperse phase in a dispersions medium, such as conceived by Nägeli (*13*); and on the other hand, the existence of only a continuous structure interpreted by Sponsler (*20*) and Astbury (*1*) in X-ray methods. Frey-Wyssling (*5*) proposed a reticular fibrillar system of structure in which long molecular chains crystallize together in some regions, and in others are not sufficiently close or regularly parallel to form a crystalline lattice. There is no corpuscular disperse phase, but both micellar and intermicellar systems are continuous, penetrating each other. Individual micelles do not exist because they are all connected with one another and grow together; the micelles of Nägeli corresponded merely to crystalline regions within a continuous, porous structure. Farr and Sisson (*4*) emphasize that the possibility of further subdivision of cellulose particles into smaller, possibly submicroscopic, crystalline masses is not obviated by their conception of membrane structure. It is likewise possible that the keratin particles may in turn be composed of submicroscopic units of structure.

All the investigators mentioned studied cellulosic fibers except Astbury (*1*), who investigated fibers of animal origin. Further analysis in the several related fields will be necessary before the exact structures of fibers and their relationships are definitely established.

The particles in wool and other animal fibers are believed to be composed of keratin since their resistance to the action of chemicals is similar to that of the entire keratinous fiber. Furthermore, like the whole fiber, the particles are doubly refractive, as are the fibrils in which they occur. These particles are spherical, having a diameter estimated at approximately 0.6μ. Nathusius (*15*), measuring dry fibrils by projection, although obtaining measurements between 0.3μ and 0.7μ, concluded that no difference in the thickness of the fibrils could be demonstrated between different kinds of hairs or between wools of different fineness. The keratin particles appear to be uniform in size, although this point is difficult to determine at the high magnifications that must be employed to see them. In different mounting media their apparent size is slightly different, as demonstrated in plate 1, *A* and *B*. However, conclusive studies along this line have not been made. The particles have a linear arrangement, producing the fibrils that have been previously recognized.

A distinctive quality of the keratin particles is their capacity to bear pigment. As early as 1867 Kölliker (*9*) called attention to the presence of granular pigment in hair. In 1894 Nathusius (*15*) discovered that the pigment granules are present in the fibrils and emphasized that they are definitely oriented structures, not mere granular deposits of pigment.

A thin transparent film has been repeatedly observed in the present investigations (pl. 1, *A*). This may be a cementing substance which holds the particles and the chains together. Moreover, the presence of such a substance between the particles might be deduced from the fact that the particles pass through the digestive tract of the carpet beetle larvae apparently without any change. Consequently, the nourishment derived from the fibers by the larvae may well be from such a cementing substance. The presence of a cementing material has long been postulated by those investigators who broke up animal fibers into their constituent cells- Nathusius (*14*, *15*), Waldeyer (*21*), and Meunier and coworkers (*11*). Waldeyer (*21*) supposed that the fibrils were differentiation products of the cellular protoplasm and that a remnant of the protoplasm was left between the fibrils as an interfibrillar cementing substance.

The presence of cellulose particles having many properties similar to the keratin particles has been reported in plant cell walls by Farr and Eckerson (*3*). More than a century ago Schleiden (*16*) and Schwann (*18*) formulated their famous cell theory, declaring the fundamental similarity of cellular structure in the plant and animal kingdoms. About 20 years later Schultze (*17*) established the protoplasm doctrine, wherein it was recognized that protoplasm is similar in all living organisms. The present writers advance the possibility that particles are the smallest visible units of mechanical structures in both plants and animals.

SUMMARY

To gain a clearer insight into the microscopical structure of animal fibers, research was carried on at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., during the years 1938 to 1940.

Minute structural units, which the writers propose to call "particles," were observed equally well in fibers subjected to the following treatments: Disintegration by chemical means; reduction to a powder by a new biological method, namely, through digestion by carpet beetles; and grinding through a special mill and using only the resulting fine dust.

The particles are composed apparently of keratin, are spherical, uniform in size, and measure approximately 0.6μ. They are doubly refractive to polarized light and sometimes bear pigment. Individual particles have been isolated and photographed.

The particles are aligned in chains, which constitute the fibrils. The chains lie parallel to the long axis of the fibers in the cortex, but they appear to twist and interlock in the medulla.

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RESPONSES OF SOME PLANTS TO EQUAL AND UNEQUAL RATIOS OF LIGHT AND DARKNESS IN CYCLES RANGING FROM 1 HOUR TO 72 HOURS[1]

By H. A. ALLARD, *senior physiologist*, and W. W. GARNER, *principal physiologist*, *Division of Tobacco Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

During the longer days of summer and in high latitudes it is a comparatively simple matter to study the length-of-day behavior of plants under the normal daylight of the natural 24-hour cycle. This can be done by the use of dark houses excluding all light, in which the plants can be kept when the daily number of hours of daylight allowed in the test each day has been satisfied. When cycles longer or shorter than the natural length of day of 24 hours are desired, the use of natural daylight supplemented with artificial light or artificial light alone must be resorted to. In the experiments now being reported upon, artificial light alone was used together with fairly constant temperature and humidity.

In the literature[2, 3, 4, 5, 6] there has been much more work reported that dealt with the natural-day cycle of 24 hours than with longer or shorter cycles, partly because specially designed and expensive equipment is required to obtain sufficiently high light intensity without excessive heating, together with accurate and dependable regulation of the desired light and darkness periods. In addition to this, control of temperature and humidity within fairly narrow limits becomes a part of the problem of the artificial climate proposed.

For this reason it has been thought desirable to present the following data for comparison with previously accumulated data based upon the same ratios in the natural-day cycle of 24 hours.[7]

MATERIAL AND METHODS

Control of ventilation, temperature, and humidity was obtained by the use of a vertical-type unit air conditioner, so adapted as to carry modified air through suitable ducts at a constant rate into eight plant cabinets, each about 19 inches square.

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[1] Received for publication March 31, 1941.

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[7] The term "cycle," as here used, refers to the time elapsing from the beginning of one light interval to another. It includes an interval of light together with the interval of darkness immediately following. In a 1 : 1 ratio of light and darkness, an alternation of 12 hours of light with 12 hours of darkness constitutes a cycle of 24 hours.

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The light in each cabinet was furnished by a specially manufactured water-jacketed and water-cooled 110-volt 1,000-watt incandescent bulb equipped with reflector, giving at a distance of 1 foot below the bowl an intensity of about 1,200 foot-candles as measured by a Weston illumination meter, model 603, equipped with a viscor filter to measure only visible radiation.

The plants were grown in porcelain sauce pots of 8-gallon capacity, 15.5 inches in diameter and 11.7 inches high, and without drainage holes at the bottom. Each cabinet held one pot.

The soil used was a well-screened, well-mixed greenhouse loam. Nine pounds of clean river sand was first leveled on the bottom of each pot, which was then filled with 85 pounds of packed soil, so that the amount of soil and sand was the same in each.

The soil placed in the cans was at the optimum tilth, the moisture content determined at this time being 19 to 20 percent.

Except in the case of *Rudbeckia bicolor*, which was first sown in flats, the seeds were sown in the containers in the warm greenhouse, and promptly at germination the seedlings were transferred to the tests. Throughout all tests temperature was maintained at 77° F., plus or minus 2°, with a humidity of 60 percent, plus or minus 2 percent.

The pots rested upon counterpoised balances in the cabinets, and the water loss was compensated for by the addition of distilled water each day.

The plants used were Peking soybean (*Soja max* (L.) Piper), a plant whose flowering is hastened by short days; *Rudbeckia bicolor* Nutt., a plant whose flowering is more favored by long days; *Zinnia angustifolia* H. B. K. (sold in the trade under the name *Z. mexicana* Hort.), a plant strictly indeterminate or day-neutral in its responses; wheat (*Triticum aestivum* L.), spring wheat variety Hard Federation, which appears to be a long-day, high-temperature type; and dill (*Anethum graveolens* L.), an umbellifer of long-day habit with respect to flowering.

In some instances a definite cycle of light and darkness was adopted, and a study of different ratios of light to darkness was made. In other instances attention was directed mainly to equal ratios of light and darkness involving total light-darkness cycles ranging from only 1 hour to as much as 72 hours. In all tests a control set was grown with alternations of 12 hours of light and darkness, to serve as the standard of behavior for the natural 24-hour cycle, which most plants experience everywhere in nature except at or near the poles, where continuous light prevails during the growing season.

EXPERIMENTAL DATA AND DISCUSSION

PEKING SOYBEAN

From the data on the Peking soybean in table 1, it is obvious that the plants grown in cabinets 1 and 2, experiencing equal alternations of light and darkness in cycles of 24 and 18 hours respectively, differed markedly in growth and behavior even though for the entire period of the experiment the total duration of illumination was the same. The 9-hour alternation somewhat delayed flowering, greatly reduced the yield of beans, increased the size of the leaves, as well as the height of the plants, and decreased the dry weight. It would appear that the shorter alternations of the 18-hour cycle tended to delay and reduce flowering, to that extent being less favorable to reproductive expression. Thus it would seem that this alternation and cycle tend toward an intensification of the long-day influence.

In cabinets 3, 4, and 5, with light periods of 4½, 6, and 7½ hours, respectively, in an 18-hour cycle corresponding to short days of 6, 8, and 10 hours in the 24-hour cycle of natural day, flowering was induced as it is when Peking soybean experiences corresponding lengths of day in these same ratios in the longer natural cycle.

When these ratios were reversed, as in cabinets 6, 7, and 8, so that the light periods were 10½, 12, and 13½ hours, corresponding to 14, 16, and 18 hours of the natural 24-hour cycle, flowering was inhibited for the entire course of the experiment. These plants consistently increased in height, dry weight, and size of leaf, as the proportion of light was increased.

It is interesting to note that the leaves grew very thick and heavy in the two cabinets (1 and 2) that received equal ratios of light and darkness, i. e., 12-hour and 9-hour alternations respectively, and that the foliage developed the same degree of greenness. In cabinets 3, 4, and 5, receiving increasing proportions of light, namely 4½, 6, and 7½ hours, respectively, the leaves developed increasingly darker greenness and the dimensions of the leaves were likewise augmented.

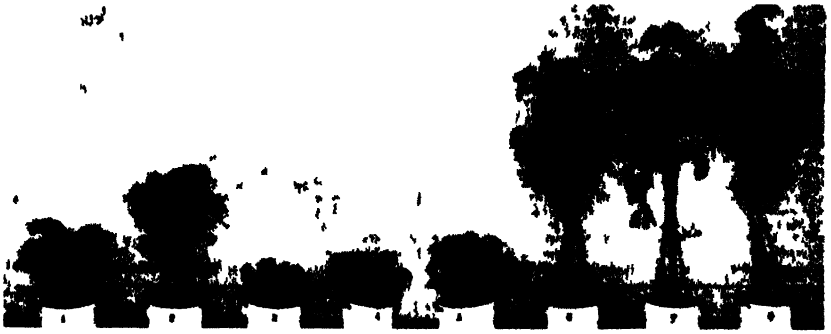

FIGURE 1.—Peking soybean (*Soja max*) grown under artificial light, with various ratios of light and darkness in cycles of 18 and 24 hours. Seed sown February 19, 1935; tests begun February 23, at germination. Plants photographed April 23, when tests ended. Numbers on pots correspond to designations of test cabinets.

1, 12 hours light, 12 hours dark. Buds March 13, flowered March 18 at 11 inches.
2, 9 hours light, 9 hours dark. Buds March 18, flowered March 22 at 17 inches.
3, 4½ hours light, 13½ hours dark. Buds March 12, flowered March 16 at 6 inches.
4, 6 hours light, 12 hours dark. Buds March 12, flowered March 16 at 7 inches.
5, 7½ hours light, 10½ hours dark. Buds March 12, flowered March 15 at 8 inches.
6, 10½ hours light, 7½ hours dark. Never budded; height when photographed, 72 inches.
7, 12 hours light, 6 hours dark. Never budded; height when photographed, 77 inches.
8, 13½ hours light, 4½ hours dark. Never budded; height when photographed, 85 inches.

With respect to the nutrition of the plants and the production of dry matter, it is obvious that increase in the hours of light, regardless of the ratio of light to darkness, resulted in improved growth. The time of flowering, on the other hand, was more affected by the ratio of light to darkness and the length of the cycle (fig. 1).

TABLE 1.—*Flowering dates, heights, green and dry weights, and other data for Peking soybean grown in an 18-hour cycle* [1]

Cabinet No	Light period	Dark period	Buds [2]	First flowers	Height of plants at—		Weight of—						Mean dimensions of terminal leaflets of 5 leaves		Leaf color and other data at end of test
					Flowering	End of test	Tops		Roots		Total tops and roots				
							Green	Air dry	Green	Air dry	Green	Air-dry	Length	Width	
	Hours	*Hours*			*Inches*	*Inches*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Inches*	*Inches*	
1 (check)	12	12	Mar 13	Mar 18	11	21	548.6	135.4	21.2	6.0	569.8	141.4	5.45	3.2	Dark green, yield of pods high, leaves thick and heavy [3]
2	9	9	Mar 18	Mar 22	17	37	598.4	114.0	30.8	7.8	629.2	121.8	6.25	4.15	Color as in 1, but yield much less, leaves very heavy, thicker than in 1
3	4½	13½	Mar 12	Mar 16	6	12	155.5	34.6	4.9	.8	160.4	35.4	4.45	2.4	Lightest green of all the tests, low yield of pods leaves thinnest of all in tests
4	6	12	do	do	7	14.5	295.5	76.3	17.6	4.0	313.1	80.3	4.2	2.45	Darker green than in 3, but not as dark green as in 1, yield of pods greater than in 3 and leaves thicker
5	7½	10½	do	Mar 15	8	19.5	370.0	89.4	19.6	4.8	389.6	94.2	5.2	3.05	About as dark green as in 4 very prolific in pods, approaching 1, leaves not as thick as in 4
6	10½	7½	None	None		72	571.0	107.9	29.2	6.9	600.2	114.8	6.1	4.2	Not as dark green as in 1 or 2, leaves thin.
7	12	6	do	do		77	936.5	145.3	39.1	8.7	975.6	154.0	7.45	5.1	About as dark green as in 6, and leaves about as thin
8	13½	4½	do	do		8[illegible]	1 116.2	187.9	59.9	11.9	1 176.1	199.8	8.05	5.5	Darker green than in 7 leaves about as thin and growth better

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[1] Tests began Feb 23, 1935, at germination, and ended Apr 23 12 plants in each pot, planted in a circle about 2 inches from the periphery

[2] Externally visible buds have been considered no microscopic examination was made in those tests where flowering did not take place

[3] Plants in cabinets 1 and 2 had leaves so thick that they broke readily when folded and pressed between the fingers, as does a mature tobacco leaf, they appeared to be packed with starch.

TABLE 2.—*Flowering dates, heights, green and dry weights, and other data for Peking soybean grown in a 36-hour cycle* [1]

Cabinet No.	Light period	Dark period	Buds	First flowers	Height of plants at—		Weight of –						Mean dimensions of terminal leaflets of 5 leaves		Leaf color and other data at end of test [2]
					Flowering	End of test	Tops		Roots		Total tops and roots				
							Green	Air-dry	Green	Air-dry	Green	Air-dry	Length	Width	
	Hours	*Hours*			*Inches*	*Inches*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Inches*	*Inches*	
1	9	27	Jan. 15	Jan. 18	9	11	98.8	18.1	4.0	0.8	102.8	18.9	2.75	1.50	Distinctly yellowish green: spindling, etiolated.
2	12	24	Jan. 18	Jan. 21	11	25	272.6	44.5	10.3	1.9	282.9	46.4	4.0	2.25	Slightly darker green than in 1.
3	15	21	Feb. 8	Feb. 12	29	39	431.6	71.0	19.6	4.0	451.2	75.0	5.25	3.00	Slightly darker green than in 2.
4	18	18	None	None		51	569.4	102.4	24.0	5.2	593.4	107.6	6.00	3.75	Slightly darker green than in 3.
5	21	15	do	do	-	48	619.0	98.1	25.5	5.6	644.5	103.7	6.00	3.75	Nearly normal green, darker than in 3.
6	24	12	do	do	-	62	802.8	126.1	27.9	5.8	830.7	131.9	7.25	4.75	Dark green, darker than in 4; stocky, leafy plants.
7	27	9	do	do	-	72	957.5	159.1	23.9	5.7	981.4	164.8	6.00	4.50	Very slightly darker than in 6; stocky, leafy plants.
8 (check)	12	12	Jan. 8	Jan. 10	11½	19	297.0	86.1	26.8	5.8	323.8	91.9	4.00	2.25	About as dark green as in 7; stocky, leafy plants.

[1] Tests began Dec. 18, 1934, at germination, and ended Feb. 18, 1935; 12 plants in each pot, planted in a circle about 2 inches from the periphery.

[2] Plants in cabinet 1 very slender and sickly. Gradual improvement shown in color and leafiness in cabinets 2, 3, 4, and 5. Plants in cabinet 5 showed evidences of slight etiolation and lack of vigor.

If the effects of equal alternations of light and darkness in a 24-hour cycle are compared with those in a 36-hour cycle (table 2), it will be noted that alternations of 18 hours of light and darkness inhibited flowering for the duration of the experiment. These plants continued to grow, reaching a height of 51 inches as compared with 19 inches for the plants receiving the 12-hour alternations, and were not as dark green as the latter. The dry weight of tops and roots of the plants in cabinet 4 somewhat exceeded the dry weight of those in cabinet 8.

In cabinets 1, 2, and 3, where light intervals were 9, 12, and 15 hours, respectively, in the ratios to darkness of 1:3, 1:2 and 1:1.4, corresponding to short days of 6, 8, and 10 hours on the basis of the natural 24-hour cycle, all the plants flowered, but with increasing delay as compared with the control plants receiving 12-hour alternations of light and darkness. With an increase in the ratio of light to darkness from 1:2 to 1:1.4 (cabinets 2 and 3), the period from germination to flowering increased from 34 to 56 days. This was attended by a marked increase in height, dry weight of plants, and size of leaves. This finding is of considerable interest, since these light durations correspond to very short days for the natural 24-hour cycle, i. e , days of 8 and 10 hours, both of which in this cycle cause earliest flowering in the Peking soybean and many other short-day plants. It would appear that with 15 hours of light in the 36-hour cycle the plants tend toward vegetative expression with retardation of flowering and consequent increase in the purely vegetative functions.

With the increase in the duration of the light intervals in the cycle of 36 hours, namely, 9, 12, 15, and 18 hours, respectively (cabinets 1 to 4), in ratios of 1:3, 1:2, 1:1.4, and 1:1, there was a gradual intensifying of the leaf green.

The plants in cabinets 5, 6, and 7 became more stocky and a darker green with increase in light intervals (21, 24, and 27 hours) in the same cycle of 36 hours, the intervals of light being longer than those of darkness and corresponding respectively to day lengths of 14, 16, and 18 hours in the 24-hour cycle. This is a natural consequence of better nutrition afforded by the increased amounts of light in the cycle.

As in table 1, the data in table 2, dealing with a single cycle of 36 hours, indicate that with increase in the hours of light there usually was an increase in dry matter produced, but as in the previous test, the time of flowering was more affected by the ratio of light to darkness (fig. 2).

The data presented in table 3 show certain interesting relations. In cabinets 1, 6, and 8, each experiencing equal alternations of light and darkness but involving cycles of 28 hours, 14 hours, and the natural cycle of 24 hours, respectively, alternations of 7 hours of light and darkness failed to cause flowering, alternations of 14 hours caused flowering in 33 days, and alternations of 12 hours caused flowering in 20 days.

At the close of the experiment the plants experiencing 14-hour alternations of light and darkness had reached a height of 50 inches; those experiencing 7-hour and 12-hour alternations had reached a height of 70 inches and 21 inches respectively. Of these three alternations, the 12-hour alternation produced the greatest amount of dry matter; the 14-hour alternation, somewhat less; and the 7-hour alternation, still less.

A peculiar feature of the development of greenness of the foliage was shown in response to the 14-hour alternation.. At first the plants were decidedly yellowish green, but this was overcome gradually until finally the plants became dark green, apparently owing to some readjustment of the chlorophyll mechanism to these light periods, a behavior at present not understood. With the 12-hour alternation, the foliage developed a normal dark greenness from the outset, while the 7-hour alternation produced at all times a lighter green.

It would appear that the plants grown under the 7-hour alternation tended rather strongly toward vegetative development, with suppression of flowering and fruiting. There was a marked increase in height by continuance of growth although the dimensions of the leaves showed only slight differences.

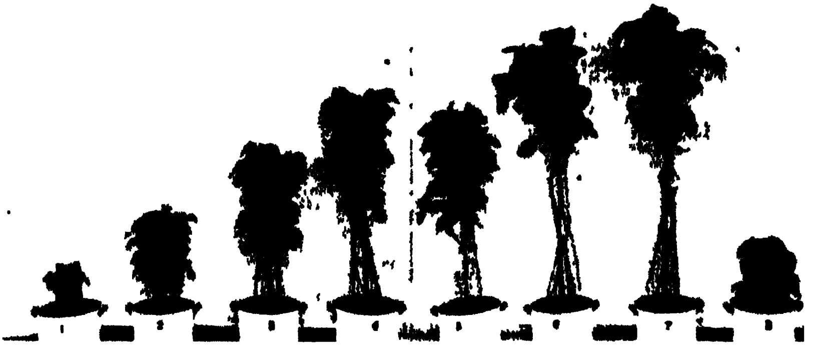

FIGURE 2.—Peking soybean (*Soja max*) grown under artificial light, with various ratios of light and darkness, in cycles of 24 and 36 hours. Seed sown December 14, 1934; tests begun December 18, at germination Plants photographed February 18, 1935, when tests ended. Numbers on pots correspond to designations of test cabinets.

1, 9 hours light; 27 hours dark. Buds January 15; flowered January 18 at 9 inches.

2, 12 hours light; 24 hours dark. Buds January 18; flowered January 21 at 11 inches.

3 15 hours light; 21 hours dark. Buds February 8; flowered February 12 at 29 inches.

4, 18 hours light; 18 hours dark. Never budded Height when photographed, 51 inches.

5, 21 hours light; 15 hours dark. Never budded. Height when photographed, 48 inches.

6, 24 hours light; 12 hours dark. Never budded. Height when photographed, 62 inches.

7, 27 hours light; 9 hours dark. Never budded. Height when photographed, 72 inches.

8, 12 hours light; 12 hours dark. Buds January 8; flowered January 10 at 11½ inches.

As in the previous tests, there was an increase in dry matter as the hours of light in the 28-hour cycle increased from 7 to 9⅓, 11⅔, 14, 16⅓, and 21 hours respectively, but time of flowering, was more affected by the particular ratios of light and darkness and also by the length of the cycle (fig. 3).

TABLE 3.—*Flowering dates, heights, green and dry weights, and other data for Peking soybean grown in cycles of 14, 24, and 28 hours*[1]

Cabinet No.	Light period	Dark period	Buds	First flowers	Height of plants at—		Weight of—						Mean dimensions of terminal leaflets of 5 leaves		Leaf color and other data at end of test
							Tops		Roots		Total tops and roots				
					Flowering	End of test	Green	Air-dry	Green	Air-dry	Green	Air-dry	Length	Width	
	Hours	*Hours*			*Inches*	*Inches*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Inches*	*Inches*	
1	14	14	Feb. 8	Feb. 10	25	50	593	114	30	6	623	120	5.5	3	Dark green; at first decidedly yellowish green; fairly productive.
2	7	21	Jan. 28	Feb. 4	8	14	119	24	5	1	124	25	3.1	1.75	Very light yellowish green; fairly productive; plants sickly from the start.
3	9⅓	18⅔	do	Feb. 3	10	16½	246	53	10.5	2	256.5	55	3.8	2.3	Somewhat darker than in 2; very productive.
4	11⅔	16⅓	do	do	11	23	426	94	21	5	447	99	5.3	3.1	Dark green; very productive.
5	16⅓	11⅔	None	None		54	651	118	32	7.5	683	125.5	6.6	4.0	Fairly dark green; very few buds.
6	7	7	do	do		70	566	80	31.5	6	597.5	86	5.5	4.0	Lighter green than in 5.
7	21	7	do	do		75	939	151.5	52.5	11	991.5	162.5	7.5	4.8	Dark normal green; most vigorous plants in the test.
8	12	12	Jan. 24	Jan. 28	9	21	476	127	27.5	7.5	503.5	134.5	5	3.1	Dark normal green, darker green than in any other cabinet; very productive in yield of beans.

[1] Tests began Jan. 8, 1936, at germination, and were concluded Mar. 10; 12 plants in each pot, planted in a circle 2 inches from the periphery.

By referring to the equal ratios of light and darkness in table 4, showing alternations of 8 hours, 16 hours, and 12 hours in cycles of 16, 32, and the natural day of 24 hours respectively, it will be seen that only the plants receiving 12-hour intervals of light flowered.

The alternations of 8 and 16 hours, on either side of the 12-hour alternations, induced a purely vegetative expression, the taller plants being produced by the 8-hour alternation. With respect to dry material produced, there was little difference, and the foliage coloration exhibited about the same shade of greenness, which was lighter than in the 12-hour plants.

In cabinets 2, 3, and 4, receiving 8, 10⅔, and 13⅓ hours of light in relation to darkness in the ratios of 1:3, 1:2, and 1:1.4, respectively, in the light-darkness cycle of 32 hours, all the plants flowered, but the flowering was delayed as the ratio of light to darkness increased or approached equality. This delay in flowering was accompanied by a consistent increase in height, in the amount of dry

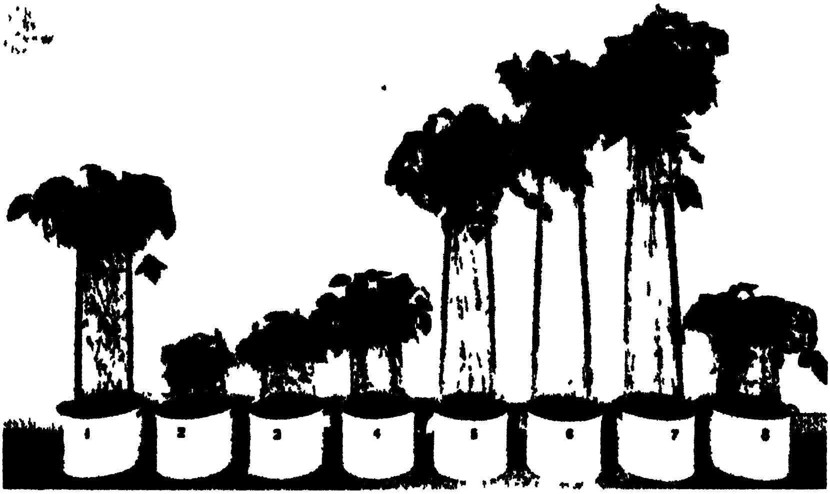

FIGURE 3. Peking soybean (*Soja max*) grown under artificial light, with various ratios of light and darkness, in cycles of 14, 24, and 28 hours. Seed sown January 4, 1936; tests begun January 8, at germination. Plants photographed March 10, when tests ended. Numbers on pots correspond to designations of test cabinets.

1, 14 hours light; 14 hours dark. Buds February 8, flowered February 10 at 25 inches. Height when photographed, 50 inches.

2, 7 hours light; 21 hours dark. Buds January 28; flowered February 4 at 8 inches. Height when photographed, 14 inches.

3, 9⅓ hours light; 18⅔ hours dark. Buds January 28; flowered February 3 at 10 inches. Height when photographed, 16½ inches.

4, 11⅔ hours light; 16⅓ hours dark. Buds January 28; flowered February 3 at 11 inches. Height when photographed, 23 inches.

5, 16⅓ hours light; 11⅔ hours dark. Never budded. Height when photographed, 54 inches.

6, 7 hours light; 7 hours dark. Never budded. Height when photographed, 70 inches.

7, 21 hours light; 7 hours dark. Never budded. Height when photographed, 75 inches.

8, 12 hours light; 12 hours dark. Buds January 24; flowered January 28 at 9 inches. Height when photographed, 21 inches.

TABLE 4.—*Flowering dates, heights, green and dry weights, and other data for Peking soybean grown in cycles of 16 and 32 hours* [1]

Cabinet No	Light period	Dark period	Buds	First flowers	Height of plants at—		Weight of—						Mean dimensions of terminal leaflets of 5 leaves		Leaf color and other data at end of test
					Flowering	End of test	Tops		Roots		Total tops and roots				
							Green	Air dry	Green	Air dry	Green	Air dry	Length	Width	
	Hours	*Hours*			*Inches*	*Inches*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Inches*	*Inches*	
1	16	16	None	None		45	594	68.5	27	5	621	73.5	6.8	4.3	About as dark green as in 6 and 7
2	8	24	Apr 20	Apr 23	9	14	137	17	12	1.5	149	18.5	3.3	1.9	Very light green, lightest green in the series
3	10⅔	21⅓	do	Apr 27	17	28	263	31.5	16	2	279	33.5	4.7	2.6	Slightly darker green than in 2
4	13⅓	18⅔	May 2	May 8	36	40	462	55	27	4.5	489	59.5	5	2.9	Slightly darker green than in 3
5	18⅔	13⅓	(2)												Test failed
6	8	8	None	None		56	556	72	31	5	587	77	5.2	3.4	Dark green, plants vigorous
7	24	8	do	do		56	840	111.5	68.2	13	908.2	124.5	7.6	4.8	About as dark green as in 6 plants vigorous
8 (check)	12	12	Apr 9	Apr 13	16	28	471	95	36	8	507	103	6.7	3.8	Darkest green in the series, productive

[1] Tests began Mar 24, 1936, at germination, and ended May 11 12 plants in each pot planted in a circle 2 inches from the periphery

[2] Plant died

material produced, and in the size of the leaflets. There was naturally an increase in the greenness of the foliage with increase in the amount of light. Plants in cabinet 2, receiving only 8 hours of light in the cycle of 32 hours, were the palest green and the most etiolated in the series. Such behavior is normal, since in the natural cycle of 24 hours this corresponds to only 6 hours of light each day, an interval of illumination that fails to give vigorous plants.

With 13⅓ hours of light and 18⅔ hours of darkness, the ratio being 1:1.4, corresponding to a 10-hour day of the natural 24-hour cycle, flowering was considerably delayed. This delay in flowering suggests a trend toward purely vegetative expression, a trend which is further indicated by the great increase in height and amount of dry material produced.

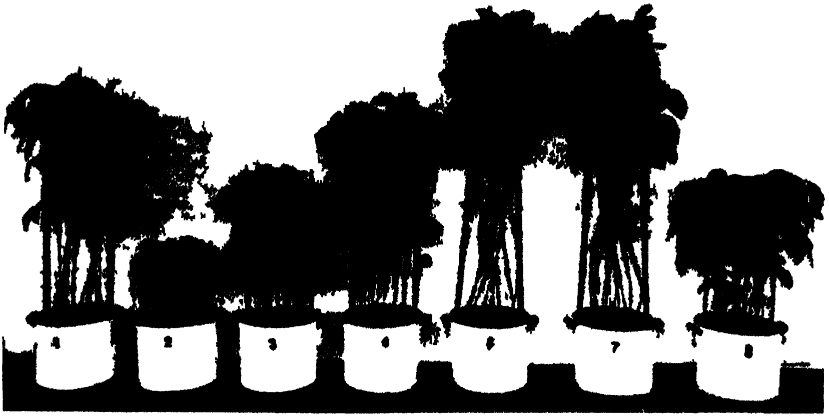

Figure 4.—Peking soybean (*Soja max*) grown under artificial light, with various ratios of light and darkness, in cycles of 16, 24, and 32 hours. Seed sown March 20, 1936, tests begun March 24, at germination Plants photographed May 11, when tests ended Numbers on pots correspond to designations of test cabinets

1, 16 hours light, 16 hours dark Never budded. Height when photographed, 45 inches

2, 8 hours light, 24 hours dark. Buds April 20, flowered April 23 at 9 inches

3, 10⅔ hours light, 21⅓ hours dark. Buds April 20, flowered April 27 at 17 inches

4, 13⅓ hours light, 18⅔ hours dark. Buds May 2, flowered May 8 at 36 inches

6, 8 hours light, 8 hours dark Never budded Height when photographed, 56 inches

7, 24 hours light, 8 hours dark. Never budded Height when photographed, 56 inches

8, 12 hours light 12 hours dark. Buds April 9, flowered April 13 at 16 inches.

There was no flowering in cabinet 7, where the light interval exceeded that of darkness in the ratio of 3:1, namely, 24 hours of light to 8 hours of darkness. This corresponds to an 18-hour day in the natural 24-hour cycle. With this increase in light, the largest yield of dry matter in the series was produced and the foliage developed a dark-green color.

As in all the previous tests with the Peking soybean, increase in the hours of light resulted in increased dry weight for the cycle of 32 hours.

Time of flowering was modified more by the ratio of light to darkness than by the length of the cycle itself (fig. 4).

HARD FEDERATION SPRING WHEAT

Table 5 shows the responses of the Hard Federation variety of spring wheat to the various light periods in cycles of 16 and 32 hours, as compared with the natural cycle of 24 hours. Referring to the equal alternations of 16, 8, and 12 hours of light and darkness in cabinets 1, 6, and 8, respectively, it will be seen that, although flowering was induced in all, the 8-hour alternations in the 16-hour cycle induced earliest flowering. Dry weights of the plants were not obtained, but the green weights were very different, the 16-hour alternations of the 32-hour cycle and the 12-hour alternations of the natural 24-hour cycle producing approximately the same green weight of material, both greatly in excess of that produced under the 8-hour alternations of the 16-hour cycle. These are differences of genuine significance, the cause of which is not as yet understood.

It is interesting to note that only two conditions in the 32-hour cycle failed to induce flowering in this wheat, namely, 8 hours of light and 24 hours of darkness, corresponding to a very short day of 6 hours of light in the natural 24-hour cycle, and 10⅔ hours of light and 21⅓ hours of darkness, corresponding to 8 hours of light and 16 hours of darkness in the 24-hour cycle, also a relatively short day in this natural cycle.

Where the ratio was increased to 13⅓ hours of light and 18⅔ hours of darkness, corresponding to 10 hours of daylight in the natural cycle of 24 hours, flowering was induced in one plant only, which produced a single head. This, then, may also be regarded as a short day unfavorable to flowering.

Flowering was induced readily when the period of light exceeded the period of darkness in a 32-hour cycle, as in cabinet 5, which received 18⅔ hours of light and 13⅓ hours of darkness, corresponding to a 14-hour day in the natural cycle of 24 hours, and in cabinet 7, which received 24 hours of light and 8 hours of darkness, corresponding to an 18-hour day in the natural 24-hour cycle. The green weight of plants was much greater in cabinet 5 than in cabinet 7, however.

A very striking feature of the behavior of the plants in cabinet 7, with the light-darkness ratio 3 : 1, corresponding to a high-latitude length of day in the natural 24-hour cycle, was the very early flowering, rapid growth, and quick maturity of the plants (fig. 5).

TABLE 5.—*Dates of heading and anthesis, green weight, and related data for spring wheat Hard Federation variety, grown in cycles of 16 and 32 hours*[1]

Cabinet No	Light period	Dark period	Heading	Pollen	Height of plants when pollen appeared	Tallest plant	Mean height of stems with heads	Tips of florets browning	Green weight of stems and roots	Average stems per plant	Average heads per plant	Average length of heads	Leaf color and other data at end of test
	Hours	*Hours*			*Inches*	*Inches*	*Inches*		*Grams*	*Number*	*Number*	*Inches*	
1	16	16	Feb 5	Feb 9	40	46 5	40 6	Feb 17	713	9	5 8	4	Very dark green vigorous
2	8	24	None	None		20	19 8		108	7 2	None		Lightest green in the series
3	10⅔	21⅓	do	do		26	23 9		317 5	10	do		Darker green than in 2
4	13⅓	18⅔	Feb 23	Feb 25	34	40	34		633	9 7	(		Dark green
5	18⅔	13⅓	Jan 25	Jan 29	43	48	42		4 8	6 6	5	4	Do
6	8	8	do	Jan 27	34	43	37 1	Feb 15	433	6 2	5 7	4	Do
7	24	8	Jan 8	Jan 13	34	41	33 8	Jan 28	213	6 8	5 1	3 5	All dry mature with yellow straw when harvested Feb 25
8 (check)	12	12	Feb 1	Feb 3	36	48	43 6	Feb 23	697	-	5 3		Dark green

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[1] Tests began Dec 14 1936 at germination and ended Feb 25 1937 about 16 plants equally spaced in each pot

[2] 1 plant with 1 head

RUDBECKIA BICOLOR

With respect to the data presented in table 6, it may be stated that *Rudbeckia bicolor* was selected as a long-day type of plant. While it is true that this species flowers more readily when afforded relatively long days in the natural cycle of 24 hours, the plants showed considerable variability in their behavior, perhaps owing to the fact that selection for this character has not been rigidly followed and the species genetically may be rather impure with respect to length-of-day responses. However, the results with this plant have been striking and very interesting. Although the plants of each cabinet received the same total duration of light for the experiment, the various alternations showed widely different effects, more especially with respect to character of growth, stem elongation, floriferous tendencies, and size of heads.

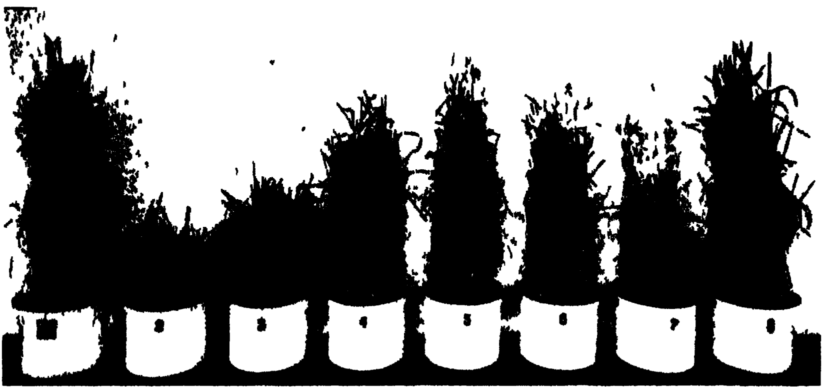

FIGURE 5.—Spring wheat (*Triticum aestivum*), Hard Federation variety, grown under artificial light, with various ratios of light and darkness, in cycles of 16, 24, and 32 hours. Seed sown December 8, 1936; tests begun December 14, at germination. Plants photographed February 24, 1937 Tests ended February 25. Numbers on pots correspond to designations of test cabinets.

1, 16 hours light; 16 hours dark. Heading February 5; pollen appearing February 9 at 40 inches.

2, 8 hours light; 24 hours dark. No heading when test ended.

3, 10⅔ hours light; 21⅓ hours dark. No heading when test ended.

4, 13⅓ hours light; 18⅔ hours dark. Heading February 23; pollen appearing February 25 at 34 inches.

5, 18⅔ hours light; 13⅓ hours dark. Heading January 25; pollen appearing January 29 at 43 inches.

6, 8 hours light; 8 hours dark. Heading January 25; pollen appearing January 27 at 34 inches.

7, 24 hours light; 8 hours dark. Heading January 8; pollen appearing January 13 at 34 inches.

8, 12 hours light; 12 hours dark. Heading February 1; pollen appearing February 3 at 36 inches.

The plants experiencing light and darkness in 12-hour alternations, a relatively short day for the natural 24-hour cycle, had produced very little stem growth with no flowers when the experiment was terminated. This behavior is normal for this plant, but there is reason to believe that flowering ultimately would have occurred, though after great delay.

TABLE 6.—*Data on flowering and growth of Rudbeckia bicolor plants grown under equal alternations of light and darkness, in 8 cycles ranging from 10 to 36 hours* [1]

Cabinet No.	Length of cycle	Light period	Dark period	Heading	First open flowers	Height at flowering	Notes on flowering and growth
	Hours	*Hours*	*Hours*			*Inches*	
1	10	5	5	Mar. 20	Apr. 6	26	Plants very floriferous; heads very large, the largest 3 inches in diameter. All plants produced stems and flowered; stems much more slender than in 2 and much less branched.
2	16	8	8	Mar. 23	Apr 14	21	Plants very floriferous; flowers generally much larger than in 1, ranging from 3 to 4 inches in diameter; stems and leaves larger, coarser, and involucral bracts larger. All plants produced stems and flowered.
3	24	12	12	None	None	12	Very little stem development. Only 2 plants produced stems; the rest remained rosettes.
4	26	13	13	Mar. 26	Apr. 25	14	Stocky plants, only 2 flowering; largest head 3.75 inches in diameter. All plants produced stems.
5	28	14	14	Apr. 8	May 3	14	Very vigorous growth; few flowers; largest head 3.75 inches in diameter. All plants produced stems
6	30	15	15	Apr. 3	Apr. 30	12	Very stocky plants, but few heads; largest head 3.75 inches in diameter. 3 plants produced stems, the rest remaining rosettes.
7	32	16	16	Apr. 8	May 3	18	Very vigorous, leafy plants; sparse flowering. Largest head, 3 inches in diameter. Only 2 plants produced stems, the rest remaining rosettes.
8	36	18	18	Mar 29	Apr 27	17	Flowering sparse; largest head, 4 inches in diameter. 4 plants with stems, the rest remaining rosettes

[1] Seed sown Jan. 8, 1937; germination Jan. 22; plants remained in warm greenhouse until tests began Mar. 2, being then only small rosettes; 8 plants in each pot, spaced 2 inches from the periphery. Experiment discontinued Apr. 30.

Plants receiving the 5-hour and the 8-hour alternations flowered most quickly and most uniformly, since all produced stems and heads. Both alternations produced very floriferous plants and very large heads. There was a striking difference in these two lots of plants, however, those receiving the 5-hour alternations producing much slenderer and less branched stems than those receiving the 8-hour alternations. The stems of the latter were much larger and bore bigger and coarser leaves. Larger flower heads were also produced, having exceptionally big, coarse involucral bracts. In the case of these two equal alternations involving cycles much shorter than the natural 24-hour cycle, it was evident that plants receiving the 8-hour alternation had much greater vegetative vigor as well as greater reproductive vigor than those receiving the 5-hour alternation.

Plants receiving equal alternations in excess of the 12-hour alternation in cabinet 3, namely 13-, 14-, 15-, 16-, and 18-hour alternations, showed a striking tendency toward delayed stem growth and floriferous expression, growing very stocky, developing a dark-green color, and producing few but large heads. Many of the plants showed no stem elongation or only slight elongation at the close of the experiment.

The most striking feature of this test was the fact that the growth behavior of the plants was greatly modified by certain alternations of light and darkness (fig. 6).

ZINNIA ANGUSTIFOLIA

Table 7 shows data on the behavior of *Zinnia angustifolia*, a typical indeterminate type of plant, in tests involving equal ratios of light and darkness of ½, 1, 2, 12, 24, 30, and 36 hours, respectively, and continuous illumination. The largest, darkest green, and earliest flowering plants were produced with continuous illumination, the plants flowering nearly 2 weeks earlier than where very short alternations of light and darkness (½ hour) were used (fig. 7).

There was a trend toward increase in dry material as the equal alternations were increased in length in cabinets 1 to 4, inclusive, involving ½-, 1-, 2- and 12-hour alternations respectively, but the weights were far below that produced by continuous illumination. With higher alternations, namely, 24, 30, and 36 hours, there was a tendency toward reduction in the dry matter produced. Alternations of 36 hours produced the smallest plants in the experiment, but with respect to greenness these were fairly dark, being scarcely lighter than the plants grown in cabinet 7 and very much darker than the light-green plants grown with ½-hour alternations (cabinet 1).

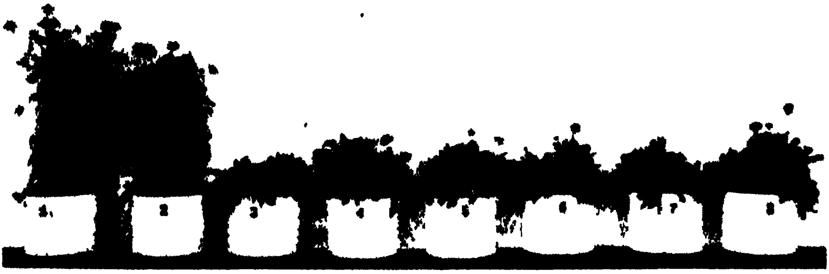

FIGURE 6.—*Rudbeckia bicolor* grown under artificial light, with equal ratios of light and darkness, in cycles of 10, 16, 24, 26, 28, 30, 32, and 36 hours. Seed sown January 8, 1937; germination January 22; tests begun March 2. Plants photographed April 30, when tests ended. Numbers on pots correspond to designations of test cabinets.

1, 5 hours light; 5 hours dark. Heading March 20; florets open April 6 at 26 inches.

2, 8 hours light; 8 hours dark. Heading March 23; florets open April 14 at 21 inches.

3, 12 hours light; 12 hours dark. No heading; stems just developing April 30, at end of test.

4, 13 hours light; 13 hours dark. Heading March 26; florets open April 28 at 14 inches.

5, 14 hours light; 14 hours dark. Heading April 8; florets open May 3 at 14 inches, after end of test.

6, 15 hours light; 15 hours dark. Heading April 3; florets open April 30 at 12 inches.

7, 16 hours light; 16 hours dark. Heading April 8; florets open May 3 at 18 inches, after end of test.

8, 18 hours light; 18 hours dark. Heading March 29; florets open April 27 at 17 inches.

Strangely enough, plants receiving 24-hour alternations were nearly as yellowish green as the plants in cabinet 1, and the longer alternations (30 hours) produced much darker green plants.

The plants in cabinets 1a to 8a, inclusive, considering first the equal alternations, showed a behavior similar to that of plants in the preceding experiment, those receiving ½-hour alternations of light and darkness having the lightest green foliage, with a gradual intensification of color as the light periods increased until continuous illumination was reached in cabinet 5a. The last-named test gave the darkest green plants in the experiment and the largest amount of dry material.

In cabinets 6a and 7a, receiving light and darkness in the ratio of 3 : 1, in cycles of 36 hours and 24 hours respectively, the time of flowering and greenness of foliage were approximately the same, but the dry matter produced was somewhat less in the case of the 36-hour cycle. With the light-darkness ratios reversed to 1:3, as in cabinet 8a, equivalent to a very short day, the plants were so badly etiolated that they were unable to flower (fig. 8).

The data presented in table 8 show the behavior of *Zinnia angustifolia* in response to equal alternations of light and darkness in cycles ranging from 10 to 36 hours.

The smallest yields of dry matter appear to have been produced with the longest alternations of light and darkness, namely, in cabinets 7 and 8, with alternations of 16 and 18 hours, respectively. Alternations of 5 hours of light and darkness in the short cycle of 10 hours produced but little more dry material. The intermediate cycles produced somewhat higher weights of dry material, but did not show particularly wide variations among themselves.

FIGURE 7. *Zinnia angustifolia* grown under artificial light, with equal ratios of light and darkness, in cycles of 1, 2, 4, 24, 48, 60, and 72 hours and with continuous light. Seed sown January 10, 1939; germination January 13. Plants photographed March 1, when tests ended Numbers on pots correspond to designations of test cabinets.

1, ½ hour light; ½ hour dark. Heading February 11; florets open February 23 at 17 inches.

2, 1 hour light; 1 hour dark. Heading February 9; florets open February 20 at 17 inches.

3, 2 hours light; 2 hours dark Heading February 6; florets open February 20 at 17½ inches.

4, 12 hours light; 12 hours dark. Heading February 2; florets open February 13 at 13 inches.

5, Continuous light. Heading January 30, florets open February 9 at 10 inches.

6, 24 hours light; 24 hours dark. Heading February 10; florets open February 23 at 15 inches.

7, 30 hours light; 30 hours dark. Heading February 6; florets open February 25 at 16 inches.

8, 36 hours light; 36 hours dark. Heading February 11; florets open February 27 at 14 inches.

The dates of flowering in all instances were remarkably close (fig. 9).

TABLE 7.—*Data on flowering, growth, green and dry weights, and related data for Zinnia angustifolia (Z. mexicana Hort.) in response to equal and unequal ratios of light and darkness* [1]

Cabinet No.	Length of cycle	Light period	Dark period	Heading	First open flowers	Height at flowering	Tallest plant at close of experiment	Weight of— Tops Green	Weight of— Tops Air-dry	Weight of— Roots Green	Weight of— Roots Air-dry	Weight of— Total tops and roots Green	Weight of— Total tops and roots Air-dry	Leaf color and other data at end of test
	Hours	*Hours*	*Hours*			*Inches*	*Inches*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	
1	1	½	½	Feb. 11	Feb. 23	17	22	196	5	19	1	215	6	Light yellowish green; lighter green than any other in this experiment. Plants slender with shorter branches.
2	2	1	1	Feb. 9	Feb. 20	17	27	326	11	34	1	360	12	Several shades darker green than in 1.
3	4	2	2	Feb. 6	do	17.5	26.5	400.5	10	40	1.5	440.5	11.5	About as dark green as in 2.
4	24	12	12	Feb. 2	Feb. 13	13	25	470	12	56	2	526	14	Do.
5	(2)	(2)	(2)	Jan. 30	Feb. 9	10	21	1,090	27.5	144.5	5	1,234.5	32.5	Darkest green plants in this experiment, with heaviest, stockiest growth.
6	48	24	24	Feb. 10	Feb. 23	15	15	322	10	29	1	351	11	Almost as light green as in 1.
7	60	30	30	Feb. 6	Feb. 25	16	16	300.5	10	27.5	1	328	11	Noticeably darker green than in 6.
8	72	36	36	Feb. 11	Feb. 27	14	14	186	5.5	17.5	.5	203.5	6	Not quite as dark green as in 7; smallest plants in experiment.
1a	1	½	½	Mar. 2	None		14.5	50	5.5	2.5	1	52.5	6.5	Very light green.
2a	2	1	1	Feb. 28	do		16.5	117	12	5	1	122	13	Somewhat darker green than in 1.
3a	4	2	2	Feb. 25	do		19	132	13.5	5	1.2	137	14.7	Somewhat darker green than in 2.
4a	24	12	12	Feb. 24	Mar. 7		18	233	22	7	1.5	240	23.5	Somewhat darker green than in 3.
5a	(2)	(2)	(2)	Feb. 21	Mar. 1		20	681	77	20	4.5	701	81.5	Darkest green of all the plants in this experiment.
6a	36	27	9	Feb. 23	Mar. 7		17	461	45	15.5	3	476.5	48	About as dark green as in 4.
7a	24	18	6	do	Mar. 2		16.5	504.5	54	19	3.5	523.5	57.5	Do.
8a	24	6	18	None	None		6.5	2	.3	.5	.1	2.5	.4	Very pale green; plants badly etiolated.

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[1] Experiments 1 to 8 inclusive began Jan. 10, 1938, and ended Mar. 1; experiments 1a to 8a inclusive began Feb. 4, 1938, and ended Mar. 9. 12 plants were grown in each cabinet.

[2] Plants in cabinets 5 and 5a received continuous illumination.

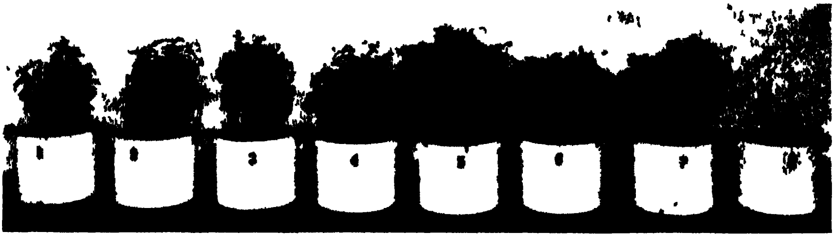

FIGURE 8.—*Zinnia angustifolia* grown under artificial light, with various ratios of light and darkness, in cycles of 1, 2, 4, 24, and 36 hours, and with continuous light. Seed sown February 1, 1938; tests begun February 4, at germination. Plants photographed March 9, when tests ended. Numbers on pots correspond to designations of test cabinets.

1, ½ hour light; ½ hour dark. Heads appearing March 2; florets never opened; height, 3½ inches when photographed.

2, 1 hour light; 1 hour dark Heads appearing February 28; florets never opened; height, 4 inches when photographed.

3, 2 hours light; 2 hours dark. Heads appearing February 25 at 4½ inches.

4, 12 hours light; 12 hours dark. Heads appearing February 24 at 5 inches.

5, continuous light. Heads appearing February 21 at 5 inches.

6, 27 hours light; 9 hours dark. Heads appearing February 23 at 5 inches

7, 18 hours light; 6 hours dark. Heads appearing February 23 at 5 inches

8, 6 hours light; 18 hours dark. Never headed; sickly, nearly died; height 3½ inches when photographed.

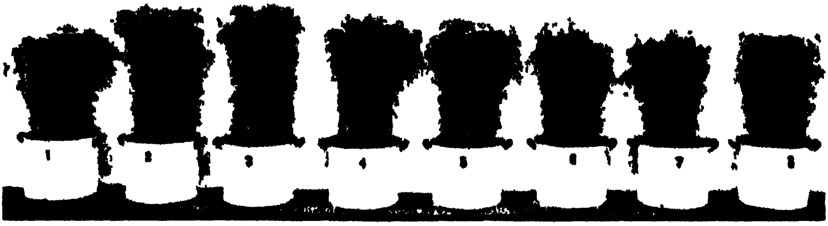

FIGURE 9.– *Zinnia angustifolia* grown under artificial light, with equal ratios of light and darkness, in cycles of 10, 16, 24, 26, 28, 30, 32, and 36 hours. Seed sown December 6, 1937; tests begun December 10, at germination. Plants photographed January 24, when tests ended. Numbers on pots correspond to designations of test cabinets.

1, 5 hours light; 5 hours dark. Heading December 31; florets open January 15 at 17 inches.

2, 8 hours light; 8 hours dark. Heading December 29; florets open January 13 at 17 inches.

3, 12 hours light; 12 hours dark. Heading December 29; florets open January 11 at 18 inches.

4, 13 hours light; 13 hours dark. Heading December 31; florets open January 14 at 17 inches.

5, 14 hours light; 14 hours dark Heading December 31; florets open January 11 at 14½ inches.

6, 15 hours light; 15 hours dark. Heading December 29; florets open January 12 at 15 inches.

7, 16 hours light; 16 hours dark. Heading December 31; florets open January 13 at 16 inches.

8, 18 hours light; 18 hours dark. Heading December 31; florets open January 15 at 14 inches

TABLE 8.—*Data on flowering, growth, green and dry weights, and related data for Zinnia angustifolia (Z. mexicana Hort.) in response to equal ratios of light and darkness in cycles ranging from 10 to 36 hours* [1]

Cabinet No	Length of cycle	Light period	Dark period	Heading	First flowers	Height of plants at flowering	Weight of—						Leaf color and other data at end of test
							Tops		Roots		Total tops and roots		
							Green	Air-dry	Green	Air-dry	Green	Air-dry	
	Hours	*Hours*	*Hours*			*Inches*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	
1	10	5	5	Dec 31	Jan 15	17	483	53.5	11.5	2	494.5	55.5	Dark green, healthy
2	16	8	8	Dec 29	Jan 13	17	719	71.5	15	2	734	73.5	About as dark green as in 1
3	24	12	12	do	Jan 11	18	507	57	10.5	2	517.5	59	Light green, slightly darker than in 8
4	26	13	13	Dec 31	Jan 14	17	547	60	15	2	562	62	About as dark green as in 1
5	28	14	14	do	Jan 11	14.5	520	61.5	11.5	2	531.5	63.5	Slightly darker green than in 3
6	30	15	15	Dec 29	Jan 12	15	516.5	64	12	2	528.5	66	Slightly darker green than in 8
7	32	16	16	Dec 31	Jan 13	16	404	46	11	2	415	48	Slightly darker green than in 5
8	36	18	18	do	Jan 15	14	466	47.5	13.5	2	479.5	49.5	Very light green, lightest green in this series

[1] Seed sown Dec 6, 1937, tests began Dec 10 at germination, and ended Jan 24 1938

DILL

The data presented in table 9 show the behavior of dill, which is a plant of long-day habit, as is clearly indicated where the plants have been given a relatively short day of 12 hours in the natural cycle of 24 hours (cabinet 4). It will be seen that these plants were considerably delayed in time of flowering, and the character of growth was inclined toward a condition of vegetativeness with respect to total dry matter produced, density of foliage, and low-branching habit.

Continuous light appreciably hastened flowering, which was followed by rapid maturity. The plants were relatively slender but very productive of seed, indicating a condition favorable to high sexual expression rather than to purely vegetative growth.

The plants grown in cabinet 1, with ½-hour alternations of light and darkness, in the very short cycle of 1 hour, were among the poorest in the experiment with respect to yield of dry matter. They were the lightest green of all the plants in this test but were apparently vigorous.

When the equal alternations of light and darkness were increased to 1 hour and to 2 hours, there was an increase in dry matter produced, together with more or less increase in height and an intensified degree of greenness of the plants, but the dates of flowering were identical with that in cabinet 1.

With the longer light-and-darkness alternations of 24 and 30 hours (cabinets 6 and 7), in cycles of 48 and 60 hours respectively, the time of flowering was delayed but the amount of dry matter was increased, as was also the height of the plants. Both conditions produced rather slender, dark-green plants, but in the case of the 30-hour alternation there was a marked increase in height of plants and dry matter produced. These results indicate that conditions in both cabinets 6 and 7 tended to be favorable to vegetative expression rather than to sexual reproduction, the latter condition being favored very obviously by continuous illumination. In other words these plants were growing under conditions more comparable to those of plants receiving 12-hour alternations in the natural-day cycle of 24 hours than to those of plants receiving the short alternations of ½ hour, 1 hour, and 2 hours, in cabinets 1, 2, and 3.

Alternations of 36 hours of light and darkness (cabinet 8) were very distinctly unfavorable to the plants, although these finally developed a nearly normal green color. The striking feature of the effects of this alternation on the plants was the extremely sickly condition shown persistently for many weeks after germination, during which period the plants made almost no growth, remaining very yellow and etiolated, and a number finally dying. As time went on, a very gradual readjustment of the chlorophyll mechanism appeared to take place, with greening of the plants and renewal of growth until they were a fairly normal green at the conclusion of the experiment. Strong main stems, even at this time, were lacking, and the plants were very short. Only one plant had budded, and flowering probably would have taken place at some very much later date. The plants at the conclusion of the experiment appeared to be directed more to the vegetative than to the reproductive state (fig. 10).

TABLE 9.—*Data on flowering, growth, green and dry weights, and related data for dill* (*Anethum gravolens*) *in response to continuous light and various equal alternations of light and darkness* [1]

Cabinet No	Length of cycle	Light period	Dark period	Buds	First flowers	Height of plants at flowering	Tallest plant at end of test	Weight of Tops Green	Weight of Tops Air-dry	Weight of Roots Green	Weight of Roots Air dry	Weight of Total tops and roots Green	Weight of Total tops and roots Air-dry	Leaf color and other data at end of test
	Hours	*Hours*	*Hours*			*Inches*	*Inches*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	
1	1	½	½	Feb 9	Feb 17	18	26	95	18	2	2	97	20	Light green, plants vigorous
2	2	1	1	do	do	23	30.5	117	23	3	2	120	25	Darker green than in 1, plants vigorous
3	4	2	2	do	do	26	42	227	40	7	3	234	43	Darker green than in 2; plants very vigorous.
4	24	12	12	Mar 4	Mar 18	60	60	810	89	35	5	845	94	Deepest green of all tests, plants very stocky, very leafy, much branched, and branched very low foliage dense
5	([2])	([2])	([2])	Feb 1	Feb 8	25	46	285	84	7	4	292	88	Growth slender, rapid, rather sparsely leafy, but vigorous and early maturing, color a healthy green
6	48	24	24	Feb 23	Mar 11	44	48	268	38	9	3	277	41	Dark green, plants rather slender
7	60	30	30	Feb 26	do	45	58	399	50	11	3	410	53	Dark green as good leafage as in 6
8	72	36	36	Mar 19	None	5	28	141	14	9	3	150	17	Plants at first very weak spindling, sickly, now showing a healthy green color no central stems would probably have flowered a week or 10 days later Only 1 plant at this time has budded

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[1] Seed sown Jan 5 1940 experiment begun Jan 10 at germination 12 plants being grown in each pot experiment ended Mar 19

[2] Plants in cabinet 5 received continuous illumination

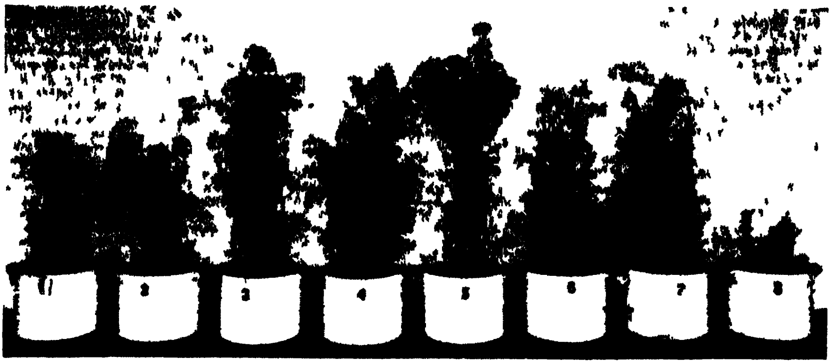

FIGURE 10. Dill (*Anethum graveolens*) grown under artificial light, with equal ratios of light and darkness, in cycles of 1, 2, 4, 24, 48, 60, and 72 hours, and with continuous light. Seed sown January 5, 1940; tests begun January 10, at germination. Plants photographed March 6. Numbers on pots correspond to designations of test cabinets.

1, Light ½ hour, dark ½ hour. Buds February 9; first bloom February 17 at 18 inches.

2, Light 1 hour; dark 1 hour. Buds February 9; first bloom February 17 at 23 inches.

3, Light 2 hours; dark 2 hours. Buds February 9; first bloom February 17 at 26 inches.

4, Light 12 hours; dark 12 hours. Buds March 4; first bloom March 18 at 60 inches.

5, Continuous light. Buds February 1; first bloom February 8 at 25 inches.

6, Light 24 hours; dark 24 hours. Buds February 23; first bloom March 11 at 44 inches.

7, Light 30 hours; dark 30 hours. Buds February 26; first bloom March 11 at 45 inches.

8, Light 36 hours; dark 36 hours. Buds March 18; no flowering when tests ended March 19.

CONCLUSIONS

From an analysis of the preceding data it is obvious that the same ratios of light and darkness afforded in different cycles, as compared with the natural-day cycle of 24 hours, have given very different results in plant behavior, depending upon the species of plant, its length-of-day requirements, and the absolute length of the cycle itself.

In certain tests the production of dry matter, which is a measure of photosynthesis and growth, increased as the light interval increased. This is clearly shown with the Peking soybean, a short-day plant, where different ratios of light to darkness were employed in the same cycle, namely, the ratios 1:3, 1:2, 1:1.4, 1.4:1, 2:1, and 3:1, thus increasing the light interval. These increases in the length of the light interval gave an actual increase in the total light received during the course of the experiment, and with more total-functioning light there may well be more growth.

In the case of equal alternations of light and darkness, it is obvious that increase in the light interval does not increase the total amount of light received during the course of the experiment. However, certain tests have shown an increase in the dry matter produced as the light interval was increased in cycles involving equal alternations of light and darkness. This would indicate complex growth relations involving the light interval itself in relation to the dark interval, since the total light and the total darkness are the same for the entire period of the test. In the case of *Zinnia angustifolia* the dry weight increased as the equal alternations increased up to 12 hours, as shown in the series involving alternations of ½, 1, 2, and 12 hours. With alternations of light intervals longer than 12 hours, however, i. e., in the series 24, 30, and 36 hours, there was a downward trend in weight of dry material produced with increase in the length of the light interval. These weights fall below those produced with alternations of 12 hours of light and darkness.

A similar behavior was shown by dill, a plant of long-day requirements, the dry weight increasing as the equal alternations increased up to 12 hours, in cycles of 1, 2, 4, and 24 hours. As in the case of *Zinnia angustifolia*, with alternations in excess of 12 hours, that is for 24, 30, and 36 hours, the weight of dry material again fell below that produced under the 12-hour alternation. The length of the cycle in this instance must somehow be concerned with growth as reflected in dry weights, in contrast to increases accompanying increased durations of light in a constant cycle.

On a purely photosynthetic basis there would be no reason to expect a longer interval of light in a series of equal alternations of light and darkness to produce a greater amount of dry material, other conditions being equal, for during the course of an experiment the summation of total light remains unchanged, yet the dry weight has changed as indicated. It would appear in these instances that a portion of the dark interval is also somehow regulative of or even inhibitory to growth and that beyond a certain length of interval this effect of the dark period dominates the situation, so that less dry matter is formed or accumulated after a certain alternation has been exceeded.

It is interesting to note that while *Zinnia angustifolia* produced by far the greatest amount of dry material with continuous illumination, this was not true of dill. The latter produced somewhat less dry matter with continuous illumination than with 12-hour alternations of light and darkness.

While it cannot at present be explained why the various ratios of light and darkness produce their characteristic effects with respect to flowering and growth of the plants, one is forced to the conclusion that the same ratios of light and darkness in different cycles may give a very different picture of plant behavior for each cycle and for each species, as to both growth and reproduction. It is apparent that not only the ratio itself is important but also the absolute cycle within which the ratio is operative for both functions. This is shown by the marked differences obtained with different cycles in time of flowering, yield of dry material, height of plants, branching, and other features of growth and specialization of the plant body.

It is unquestionably true also that the fundamental physiology of the mechanism of metabolism and growth is sometimes affected adversely soon after germination, and that this effect is followed sooner or later by more or less complete readjustment to the unusual conditions. This has more than once been shown by a marked etiolation and sickliness of the plants at the outset, on certain equal alternations, with a gradual recovery and a return to fairly normal growth and greenness as time went on and more leafage was produced.

It would appear that perhaps an increase in leaf area is one of the factors concerned in this striking recovery.

SUMMARY

A study has been made of the responses of some plants to various photoperiods under artificial light alternating with darkness in various ratios. Continuous illumination also was used. Light was furnished by water-jacketed and water-cooled 110-volt, 1,000-watt incandescent bulbs equipped with reflector. One bulb was used in each cabinet, the intensity at 1 foot from the plants being about 1,200 footcandles.

Temperatures were maintained at approximately 77° F., with a relative humidity of about 60 percent, in all tests. The pots rested on counterpoised balances, and distilled water was added to compensate for water loss each day.

The ratios of light to darkness used were 1:3, 1:2, 1:1.4, 1:1, 1.4:1, 2:1, and 3:1. The standard adopted for control was 12 hours of light alternating with 12 hours of darkness, a ratio of 1 : 1 in a cycle of 24 hours, which is the natural cycle in most latitudes of the earth. Cycles as short as 1 hour and as long as 72 hours were used.

The plants studied were Peking soybean (*Soja max* (L.) Piper), *Zinnia angustifolia* H. B. K., Hard Federation variety of spring wheat (*Triticum aestivum* L.), *Rudbeckia bicolor* Nutt., and dill (*Anethum graveolens* L.). On the basis of the natural 24-hour cycle, Peking soybean is usually regarded as a plant whose flowering is favored by short days; *Zinnia angustifolia* is obviously indeterminate, or day-neutral, in its flowering relations to length of day; spring wheat and *Rudbeckia bicolor* are favored by long days with respect to flowering; and dill is likewise a long-day type of plant.

The outstanding result of the experiments was that in most cases the same ratios of light and darkness did not give the same behavior for any particular plant.

In the case of Peking soybean, a short-day plant, a 1:1 ratio of light and darkness produced ready flowering with 12-hour alternations. With 7- and 8-hour alternations flowering was inhibited; with 9- and 14-hour alternations flowering was delayed; and with 16- and 18-hour alternations, flowering was again inhibited. Increase in the interval of light afforded the plants in any given cycle resulted in increased growth and yield of dry matter. The time of flowering was dependent upon both the ratio of light to darkness and the length of the cycle.

The results secured with *Zinnia angustifolia* and dill, subjected to equal alternations of light and darkness in cycles ranging from 1 to 72 hours, showed more or less obvious differences in flowering behavior and growth characteristics. In the case of the former, a strictly indeterminate plant, time of flowering was less affected; but the character of growth, the dry matter produced, and the greenness of the foliage were markedly different. Dill, a long-day plant, showed marked differences in time of flowering as well as in height, dry matter produced, branching habit, and shade of greenness.

In addition to equal ratios of light and darkness, unequal ratios were also studied, including 1:3, 1:2, 1:1.4, and the reverse of these, in different cycles. All produced characteristic effects, depending upon the species of plant, its length-of-day requirements, and the absolute length of the cycle used.

The results of the work herein reported indicate that increase in growth and the production of dry matter may be associated with increase of the light interval in two contrasting conditions. In the one the dry matter increased where the total light received during the course of the experiment was also increased, as in the case of unequal ratios of light and darkness in the same cycle. In the other, the dry matter increased where equal ratios of light and darkness were maintained in cycles increasing in length, that is, where the total amount of light remained the same during the course of the experiment.

INHERITANCE OF SUSCEPTIBILITY TO INFECTION BY HELMINTHOSPORIUM MAYDIS RACE 1 IN MAIZE[1]

By Arnold J. Ullstrup

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INTRODUCTION

In an earlier paper[2] *Helminthosporium maydis* Nisik. and Mke. was reported as occurring on dent corn (*Zea mays* L.) in Indiana, Illinois, and Ohio. The fungus is known to attack suscepts at all stages of development. Two morphologically indistinguishable races of the species were recognized on the basis of host specialization, symptoms produced, and relative virulence. It was proposed that *H. maydis* race 1 include the parasite which thus far has been limited in its parasitism to the one inbred line *Pr*. All other inbred lines and hybrids, including single crosses involving *Pr*, observed in the field or tested in the greenhouse proved to be highly resistant to this race. This paper reports the results obtained in a study of the inheritance of susceptibility to the disease caused by *H. maydis* race 1.

MATERIALS AND METHODS

Plants of three single crosses involving *Pr*, namely, *Pr* × *Hy*, *Pr* × 38 11, and *Pr* × *Tr*, were grown in the field in 1939, and their ears were selfed or backcrossed with pollen of *Pr*, the susceptible parent, to obtain F_2 and backcrossed seed respectively. F_2 seed produced in the greenhouse the previous winter from F_1 plants of the same single crosses was planted in the field and self-pollinated to obtain F_3 seed.

Seed of F_2, backcrossed, and F_3 progenies was planted in flats in the greenhouse during the following winter. When the seedlings attained the 3- to 4-leaf stage they were sprayed on two successive occasions with a spore suspension of *Helminthosporium maydis* race 1. After each application of inoculum to the plants the flats were placed in moist chambers for 15 to 17 hours. Preliminary trials indicated that this length of time in a moist chamber was sufficient to initiate infection and did not cause water soaking and subsequent necrosis of the tissues such as frequently occurs when corn seedlings are held in a saturated atmosphere for an extended period. Seedlings of the susceptible inbred line and seedlings of the resistant single crosses were placed in each moist chamber to serve as controls.

Inoculum was prepared in the following manner. A single ear of *Pr* known to be infected with *Helminthosporium maydis* race 1 was

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[1] Received for publication December 18, 1940. Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Department of Botany, Purdue University Agricultural Experiment Station

[2] ULLSTRUP, ARNOLD J. TWO PHYSIOLOGIC RACES OF HELMINTHOSPORIUM MAYDIS IN THE CORN BELT. Phytopathology 31 508-521, illus 1941

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used as a stock culture. Prior to inoculating a seedling population a few kernels were removed from this ear, surface-sterilized, and plated on acidified potato-dextrose agar. When sufficient growth of the fungus was visible on the plates, transfers were made to several fresh plates of potato-dextrose agar. When the growth had covered the agar surface the spores were washed off in water and the suspension was transferred to an atomizer and sprayed on the seedlings. The ear of *Pr* infected with the fungus was used as a stock culture

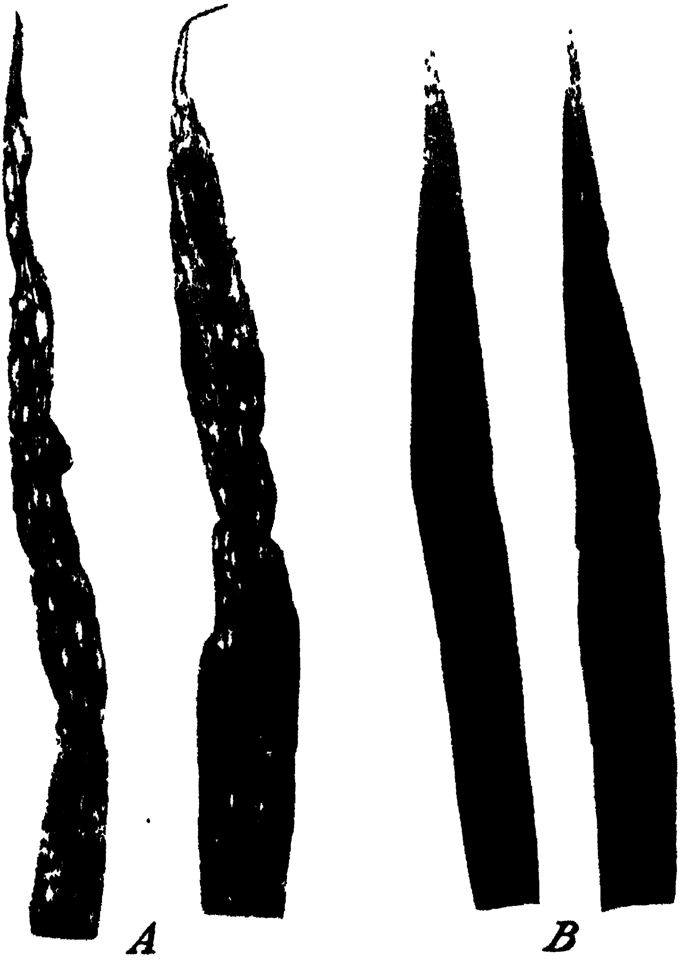

FIGURE 1.- *A*, Pair of leaves of a susceptible segregate showing typical symptoms; *B*, pair of leaves of a resistant segregate showing minute flecks where penetration has taken place, but progress of the parasite has been arrested.

in preference to a culture maintained on agar in order to avoid the possibility of inadvertent selection of nonpathogenic variants that frequently arise in artificial culture. Inoculum prepared in this way has always given consistent results on segregating seedling populations.

Symptoms developed rapidly, and 7 days after the last inoculation the seedlings were classified according to their reaction. Resistance and susceptibility are clear-cut (fig. 1), and no difficulty was experienced in determining the class to which a seedling belonged.

RESULTS

The data on the F_2 seedling segregations of the three original crosses are shown in table 1. Out of a total of 1,766 seedlings tested, 1,325 were resistant and 441 were susceptible. The deviation from a theoretical 3:1 ratio is not significant. In table 2 the results of the test of seedlings of the backcrosses are given. A total of 1,746 seedlings showed a segregation of 882 resistant and 864 susceptible. The deviation from a 1:1 ratio is without significance. The segration of F_3 families is presented in table 3. In a total of 275 families tested, 74 were resistant, 141 were segregating, and 60 were susceptible. The deviation in this case from a 1:2:1 ratio is not statistically significant and is attributable to sampling error.

TABLE 1. *Segregation of resistant and susceptible seedlings in the F_2 populations from 3 crosses involving the inbred line Pr*

[100 kernels planted of each F_2 progeny]

Parentage	Resistant	Suscep-tible	Deviation from a 3:1 ratio	Deviation / Standard deviation
(*Pr* × 38-11)-1	78	22	3.00	0.69
(*Pr* × 38-11)-2	81	19	6.00	1.38
(*Pr* × 38-11)-3	72	28	3.00	.69
(*Pr* × 38-11)-4	80	20	5.00	1.15
(*Pr* × 38-11)-5	77	21	3.50	.82
(*Pr* × 38-11)-6	69	30	5.25	1.22
Total	457	140	9.25	.87
(*Pr* × *Hy*)-1	69	25	1.50	.36
(*Pr* × *Hy*)-2	72	28	3.00	.69
(*Pr* × *Hy*)-3	71	28	3.25	.75
(*Pr* × *Hy*)-4	74	24	.50	.12
(*Pr* × *Hy*)-5	73	27	2.00	.46
(*Pr* × *Hy*)-6	71	27	2.50	.58
Total	430	159	11.75	1.12
(*Pr* × *Tr*)-1	75	23	1.50	.35
(*Pr* × *Tr*)-2	76	20	4.00	.94
(*Pr* × *Tr*)-3	72	26	1.50	.35
(*Pr* × *Tr*)-4	75	20	3.75	.89
(*Pr* × *Tr*)-5	70	26	2.00	.47
(*Pr* × *Tr*)-6	70	27	2.75	.64
Total	438	142	3.00	.29
Grand total	1,325	441	.50	.03

The above results indicate that in the crosses studied resistance and susceptibility to *Helminthosporium maydis* race 1 are conditioned by a single pair of genes. It is suggested that these genes be designated as *Hm hm*. In heterozygous individuals resistance is completely dominant. The inbred line *Pr* is the only known source of the recessive allelomorph for susceptibility. No crosses studied thus far indicate other than typical monogenic inheritance.

TABLE 2.—*Segregation of resistant and susceptible seedlings in the backcrossed progenies from 3 single crosses involving Pr*

[100 kernels planted from each backcrossed ear]

Parentage	Resistant	Susceptible	Deviation from a 1 1 ratio	Deviation / Standard deviation
(*Pr* × 38-11) × *Pr*-1	47	52	2 5	0 50
(*Pr* × 38 11) × *Pr*-2	47	52	2 5	50
(*Pr* × 38-11) × *Pr*-3	51	49	1 0	20
(*Pr* × 38-11) × *Pr*-4	52	46	3 0	61
(*Pr* × 38-11) × *Pr*-5	44	55	5 5	1 01
(*Pr* × 38-11) × *Pr*-6	50	48	1 0	20
Total	291	302	5 5	45
(*Pr* × *Hy*) × *Pr*-1	49	45	2 0	41
(*Pr* × *Hy*) × *Pr* 2	49	50	5	10
(*Pr* × *Hy*) × *Pr*-3	54	41	6 5	1 33
(*Pr* × *Hy*) × *Pr*-4	46	51	2 5	51
(*Pr* × *Hy*) × *Pr*-5	50	50	0	00
(*Pr* × *Hy*) × *Pr*-6	42	47	2 5	53
Total	290	284	3 0	25
(*Pr* × *Tr*) × *Pr* 1	50	45	2 5	51
(*Pr* × *Tr*) × *Pr* 2	50	44	3 0	64
(*Pr* × *Tr*) × *Pr* 3	45	50	2 5	51
(*Pr* × *Tr*) × *Pr* 4	54	45	4 5	90
(*Pr* × *Tr*) × *Pr*-5	50	47	1 5	30
(*Pr* × *Tr*) × *Pr*-6	52	47	2 5	50
Total	301	278	11 5	95
Grand total	882	864	9 0	43

TABLE 3 -*Segregation of F_3 families*

[25 kernels planted of each family]

arental cross	Number of F_3 families tested	Observed segregation of families			Theoretical segregation on 1 2 1 basis			χ^2	Range of *P*
		Homozygous resistant	Segregating	Homozygous susceptible	Homozygous resistant	Segregating	Homozygous susceptible		
8 11)	83	20	46	17	20 75	41 50	20 75	1 19	0 50-0 70
(*Pr* × *Tr*)	50	9	29	12	12 50	25 00	12 50	1 64	30- 50
(*Pr* × *Tr*)	53	17	23	13	13 25	26 50	13 25	1 53	30- 50
(*Pr* × *Hy*)	89	28	43	18	22 25	44 50	22 25	2 35	30- 50
otal	275	74	141	60	68 75	137 50	68 75	1 60	30 50

SUMMARY

The dent corn inbred line *Pr* is unique in its susceptibility to infection by *Helminthosporium maydis* race 1. All other corn inbred lines and hybrids, including single crosses involving *Pr*, tested in the greenhouse or observed in the field proved to be highly resistant.

The susceptibility of the inbred line *Pr* to infection by *H. maydis* race 1 is inherited as a monogenic recessive. The pair of genes concerned have been designated as *Hm hm*.

EFFECT OF STAGE OF MATURITY AT TIME OF HARVEST ON GERMINATION OF SWEET CORN [1]

By C. W. CULPEPPER, *physiologist*, and H. H. MOON, *assistant pomologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture* [2]

INTRODUCTION

Several workers (*1*, *2*, *3*)[3] have shown that the quality of sweet corn as a table product is greatly influenced by its stage of maturity when harvested. The stage for optimum quality is generally considered to be that at which the grain has reached one-half to three-fourths of its full maturity as judged by its dry-matter content.

It has been shown by Straughn and Church (*7*) that there is little relationship between the sugar content of the grain at full maturity and the sugar content at the time when the table quality is maximum. It is thus obvious that it would be advantageous to make selections of desirable individual plants upon the basis of the characteristics of the grains at the edible stage rather than at full maturity. Such a procedure would be greatly facilitated if the ears could be harvested when in optimum condition for table use, a portion of each used for making the necessary tests for quality, and the remainder preserved for planting.

The question immediately arises as to how the premature harvesting of such material will affect its germinating power when subsequently used for seed. If the seeds germinate, will the subsequent growth and behavior of the plants be affected adversely? If so, to what extent? How immature can sweet corn be when harvested and yet possess ability to germinate and develop to maturity? How are viability and capacity for normal development related to composition and size of the developing grain? Are there differences in varieties? If so, how may these differences be explained? While several of these questions have been answered for field corn and some other grains, no extensive study of the behavior of sweet corn in particular has been made.

Certain varieties, such as Golden Bantam and Stowell Evergreen, appear to mature at different rates. Is there any relation between rate of maturity and viability of immature grains?

After the grain has reached its full development, what is the effect of delayed harvest on the viability under the field conditions that prevail in the warm, humid parts of the eastern United States?

Information bearing upon these questions will be of particular value to the geneticist and the plant breeder.

A number of workers have presented reports concerned with the effect of stage of maturity upon the germination of the seeds of several grain crops. Robinson (*5*), using two varieties of field corn, has made a rather comprehensive study of the physiological factors

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[2] The authors are indebted to D. N. Shoemaker, formerly of the Division of Fruit and Vegetable Crops and Diseases, for advice and suggestions in making the germination tests.

[3] Italic numbers in parentheses refer to Literature Cited, p. 359.

affecting germination. Sprague (*6*) has studied the effect of various factors on the dormancy, after-ripening, and germination of seed corn when harvested at different degrees of maturity. These writers have given a rather complete bibliography of the work done with corn and other grains, consequently no further review of the literature will be given here. Neither of these workers, however, dealt specifically with sweet corn; and it would be expected that, because of the wrinkled and tender character of the seed, sweet corn might behave differently from field corn and other grains.

MATERIAL AND METHODS

The sweet corn for the tests reported in this paper was grown at the Arlington Experiment Farm, Arlington, Va., on a sandy loam soil formed by dredging operations along the Potomac River. The plants generally made good growth and were characteristic of the respective varieties.

In two seasons the ears for the tests were tagged when the silks first appeared, and the samples for the tests were harvested at predetermined ages. The ears were pollinated naturally in two seasons, but in a third season the pollinations were made by hand in order to obtain an accurate record of the date of fertilization. Samples were generally taken at 1- or 2-day intervals from 5 to 25 days from date of silking. Beyond the 25-day stage the samples were taken at 5-day intervals.

The ears were harvested about 9:30 a. m. and the husks removed. A portion of the ears was dried with the grains on the cob, and from the remainder the grains were removed from the cob by cutting just below the base of the grain with a sharp knife. In some of the work the grains from one-half of each ear were removed from the cob and the remainder dried without so removing. The material for the germination tests was then dried in a constant-temperature room at 26° C. in a current of air from an electric fan. The length of time required to bring the seed to an air-dry condition (moisture content 10 to 12 percent) varied with the atmospheric humidity, the size of the grain, and the degree of maturity. Four to seven days were generally required to dry material cut from the cob, and 6 to 10 days to dry that on the cob. Varieties like Stowell Evergreen required longer than varieties like Golden Bantam. In some cases effort was made to vary the conditions so that ears of different sizes would dry in the same length of time. The humidity of the room was not measured, but the atmosphere was sufficiently dry to be unfavorable for the development of micro-organisms, and consequently the corn remained free from any signs of decay or fermentation during or subsequent to the drying process.

Immediately after cutting from the cob, samples of the material were taken for chemical analysis.

After being dried, the samples were stored until the following January, at which time the germination tests were made. Material for each germination test was obtained from a lot of 5 to 10 ears by shelling 2 rows of grains from opposite sides of each ear and combining the grains obtained. From this material, duplicate samples of 100 grains each were drawn, care being exercised to include grains from tip and base of the ears as well as from the middle region. These duplicate samples were then planted in clean sand in the greenhouse The time at which the plants appeared above ground was noted, and at the end of 2 weeks the plants were removed from the sand and the ungerminated seeds examined for presence or absence of decay.

In the first year the Golden Bantam and Stowell Evergreen varieties were used, both being planted on the same date. The Golden Bantam came into flower about 10 days earlier than the Stowell Evergreen, and there was a corresponding difference in the time of harvest. Harvesting of the Golden Bantam was continued at 1- to 5-day intervals until the series from both varieties was completed.

In the second year several plantings were made at such dates that flowering in the two varieties occurred at the same time. The harvesting of the samples for each age was done on the same date for the two varieties, to eliminate differences in the climatic conditions during the period while the ears were developing. In drying the material the Golden Bantam ears were placed at a somewhat greater distance from the electric fan than the Stowell Evergreen, so that the two varieties were brought to air-dry condition in about the same length of time.

RESULTS OF FIRST AND SECOND YEARS' TESTS

WEIGHT, DRY MATTER, SUGAR CONTENT, AND GERMINATION

The results of the first and second years' tests are given in table 1. The air-dry weight per grain of the young material is doubtless a little high, as a certain amount of chaffy material adhered to the base of the grains and could not be removed quickly without injuring the rather fragile pericarp. The very young material when quite dry was rather brittle and delicate, so that it was always necessary to handle it with considerable care. The dry-weight determinations of the older grains are much more exact.

It is to be noted that the percentage of germination between the 11- and the 15-day stages of maturity is very low and irregular in both years. After the 15-day stage the percentage of germination increased strikingly to the 20-day stage, at which time about 80 to 90 percent germinated.

The material cut from the cob at the time of harvest germinated less satisfactorily than that which was allowed to remain on the cob. This result may have been due in part to injury to the grain in cutting but was probably due for the most part to the fact that the grains remaining on the cob derive some sustenance from it during drying.

There is a good correlation between the percentage of dry matter in the grain at the time of harvest and the percentage of grains germinating. With dry matter below 18 percent for corn cut from the cob or 14 to 17 percent for that dried on the cob, germination was very poor; but with dry matter above 20 percent, germination was generally good whether the grains had been dried on or off the cob.

The power of germination does not seem to be directly related to the sugar content. The germination increased with age up to 22 to 25 days, but the sugar content increased to about the 16- or 18-day stage and then decreased. Thus, satisfactory germination was not obtained until the sugar content had declined considerably from its maximum.

TABLE 1.—*Air-dry weight, dry-matter content, sugar content, and germination of seeds of the sweet corn varieties Golden Bantam and Stowell Evergreen, harvested at different stages of maturity, in first and second years' tests*

GOLDEN BANTAM

Age from date of silking (days)	Air dry weight per seed				Dry matter at harvest		Sugar content at harvest		Germination of seeds in greenhouse			
	First year		Second year		First year	Second year	First year	Second year	First year		Second year	
	Dried on cob	Dried off cob	Dried on cob	Dried off cob					Dried on cob	Dried off cob	Dried on cob	Dried off cob
	Gram	*Gram*	*Gram*	*Gram*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
8						9.41		3.57	0	0	0	0
9			0.0113						0	0	0	0
10	0.0181	0.0172	.0113	0.0108	10.42	8.87	3.62	3.04	0	0	4	0
11	.0312	.0291		.0122					0	0	2	0
12	.0356	.0311	.0290	.0221	12.69	11.62	4.71	4.53	1	0	25	0
13	.0471	.0420	.0265	.0292					6	0	6	1
14	.0636	.0662	.0425	.0296	14.80	12.14	6.03	6.12	6	2	55	0
15	.0697	.0668	.0533						10	6	65	0
16	.0894	.0714	.0564	.0484	18.36	14.48	6.55	5.94	88	4	76	25
17	.0904	.0861	.0677	.0545					90	23	75	12
18	.0992	.0921	.0848	.0625	20.94	19.10	5.10	4.97	96	44	82	19
19	.1181	.1099	.0940	.0840					97	39	91	45
20	.1275	.1298	.1008	.0855	23.24	21.64	4.12	4.12	94	46	77	78
22	.1353	.1314	.1248	.1053	27.41	25.16	4.00	3.90	99	85	97	96
25	.1511	.1561	.1459	.1288	33.96	30.13	3.61	2.47	98	87	100	99
30	.1812	.1841	.2022	.2044	40.85	36.64	1.85	1.54	99	87	98	100
35			.2369	.2212		42.32					99	100

STOWELL EVERGREEN

Age from date of silking (days)	Air dry weight per seed				Dry matter at harvest		Sugar content at harvest		Germination of seeds in greenhouse			
	First year		Second year		First year	Second year	First year	Second year	First year		Second year	
	Dried on cob	Dried off cob	Dried on cob	Dried off cob					Dried on cob	Dried off cob	Dried on cob	Dried off cob
						9.14		3.29	0	0	0	0
10	0.0118	0.0100	0.0140	0.0143	10.12	9.01	4.12	3.18	0	0	0	0
11	.0128	.0119	.0093	.0129					1	0	2	0
12	.0124	.0125	.0101	.0156	11.46	10.64	4.36	4.59	4	2	0	0
13	.0168	.0154	.0133	.0135					20	1	4	0
14	.0187	.0185	.0291	.0226	13.77	12.32	4.92	4.32	40	4	24	[illegible]
15	.0236	.0200	.0320	.0243					25	20	61	0
16	.0287	.0246	.0398	.0345	17.16	14.72	6.51	5.87	74	[illegible]	55	[illegible]
17	.0374	.0381	.0432	.0400					79	49	66	20
18	.0528	.0506	.0531	.0514	19.02	17.21	5.39	5.76	90	55	74	21
19	.0702	.0691	.0664	.0648					97	80	72	42
20	.0804	.0812	.0804	.0773	21.30	19.27	4.29	4.52	98	80	88	59
22	.0864	.0834	.1043	.1054	26.14	22.64	3.87	3.85	99	88	88	94
25	.0955	.0901	.1301	.1184	27.44	24.82	3.45	3.02	98	93	99	95
30	.1634	.1602	.1971	.1740	32.18	29.77	2.65	[illegible]	100	91	100	100
35	.2150	.2190	.2041	.1822	36.71	36.32	1.60	1.71	100	98	97	98

It was expected that, since the Golden Bantam appeared to mature more rapidly than the Stowell Evergreen, it would also germinate at a somewhat earlier age. In the material cut from the cob there appeared to be no very significant difference between the varieties, but in the material allowed to dry on the cob the Stowell Evergreen germinated considerably better than the Golden Bantam the first year, whereas the opposite occurred the second year. There is no clear explanation for this reversal in behavior. The two varieties were not harvested at exactly the same dates. The differences may possibly have been due to differences in the growing conditions prevailing in the period between silking and harvest of the two varieties.

The material of Stowell Evergreen cut from the cob germinated somewhat better in the first year than in the second, but in Golden Bantam the differences were not consistent.

BEHAVIOR OF IMMATURE GRAINS WHEN PLANTED IN THE FIELD

The young plants germinated in the greenhouse in the above tests showed tremendous differences in size and vigor that are not revealed by the percentages of germination. The size of the foliage as it appeared above ground was generally smaller the earlier the material was harvested. The plants from the seed harvested very young had the appearance of rye or wheat, and the very youngest appeared even as diminutive as some small-seeded grass. This, of course, raised the question whether such material when planted in the field would survive and develop to maturity.

A quantity of seed of the second crop harvested at different stages of maturity and dried without cutting from the cob was planted in the field in the following June. It was planted much more thickly than is ordinarily done. The conditions in the field were very favorable for germination, and in all except the very immature lots thinning was necessary to permit normal development of the plants. None of the plants from the seed harvested below 15 days of age survived to make a normal growth. The plants from the immature seeds appeared to be somewhat slower in getting established than those from the normal grain, but when once established they apparently grew as fast as the plants from the mature seed. The plants from the seed harvested at 15 to 17 days of age came into flower 3 to 6 days later than the plants from the mature seed.

There was very little difference in the date of flowering of any plants from seed harvested at 20 days of age or more, although the weight of the seeds varied greatly between the 20- and 30-day stages. It appeared quite possible to grow a crop satisfactorily in the field from seeds harvested 20 days or more after pollination. Of course, the minimum safe age for harvesting seed would depend upon the climatic conditions prevailing during the development of the grains, the required age at time of harvest being greater for material developing during the cooler part of the growing season or in northern sections.

THIRD YEAR'S STUDIES

In the third year's work all pollinations were made by hand in an attempt to get more uniform results. It had been suspected that the irregular results of the first 2 years' germination tests were due in part to the younger age of the grains near the base and tip of the ear than those near the middle of the ear, which are naturally fertilized first. The ears were bagged a few days before the silks appeared, and 2 days after the silks first appeared the ears were pollinated with fresh pollen from the same variety as the ear that was being pollinated. The ears were kept bagged until harvested. The pollinations were made between 9 and 11 a. m., and the ears were harvested at the same period of the day at 2-day intervals beginning the day after pollination.

WEIGHT, DRY MATTER, SUGAR CONTENT, AND GERMINATION

In addition to testing grain dried with and without cutting from the cob, another series was taken by harvesting the entire aerial portion of the plant, bringing it into the laboratory, and allowing it to dry at ordinary room temperature until the outer husks became almost air-dry, after which the ears were removed from the stalks, the husks removed, and the drying completed in the constant-temperature room under the electric fan. Twelve to eighteen days, depending on the age of the material, was required to complete the drying of the grains. The results of these tests are given in table 2

The percentage of grains germinating was more consistent from day to day than in the previous years, but there still remained considerable irregularity that must be attributed to causes other than differences in the age of the grains from pollination

Differences between varieties appear too small to be of any particular importance

TABLE 2.—*Air-dry weight, dry matter, sugar content, and germination of seeds of the sweet corn varieties Golden Bantam and Stowell Evergreen, harvested at different stages of maturity, in third year's tests* [1]

GOLDEN BANTAM

Age from date of pollination (days)	Air dry weight of grain: Ear dried on stalk	Air dry weight of grain: Ear removed from stalk but grains dried on cob	Air dry weight of grain: Grains dried after cutting from cob	Dry matter at harvest	Sugar content at harvest	Germination: Ear dried on stalk	Germination: Ear removed from stalk but grains dried on cob	Germination: Grains dried after cutting from cob
	Gram	*Gram*	*Gram*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
2						0	0	0
4				10.10	3.40	1	0	0
6				9.84	3.56	2	1	0
8	0.0422	0.0361	0.0284	10.72	4.50	10	0	0
10	.0497	.0405	.0217	12.30	4.65	20	12	0
12	.0701	.0572	.0458	14.58	5.32	70	49	2
14	.0785	.0764	.0642	16.87	6.43	90	52	42
16	.1045	.0894	.0872	20.25	5.59	96	98	50
18	.1237	.1079	.1040	23.28	4.34	100	100	87
20	.1504	.1398	.1326	29.17	3.51	97	100	87
25	.1842	.1662	.1672	36.49	2.12	98	100	100
30	.2146	.2095	.1996	44.22	1.49	99	100	100

STOWELL EVERGREEN

Age from date of pollination (days)	Air dry weight of grain: Ear dried on stalk	Air dry weight of grain: Ear removed from stalk but grains dried on cob	Air dry weight of grain: Grains dried after cutting from cob	Dry matter at harvest	Sugar content at harvest	Germination: Ear dried on stalk	Germination: Ear removed from stalk but grains dried on cob	Germination: Grains dried after cutting from cob
2						2	0	0
4				9.73	3.81	0	0	0
6	0.0336	0.0235	0.0167	9.16	3.64	4	0	0
8	.0314	.0229	.0248	10.43	3.72	3	0	0
10	.0378	.0296	.0187	12.06	4.53	30	5	0
12	.0532	.0419	.0290	13.91	5.12	69	39	0
14	.0867	.0735	.0402	16.39	6.24	79	47	40
16	.1015	.0910	.0498	19.47	4.97	98	90	81
18	.1342	.1234	.0841	22.12	4.00	100	98	85
20	.1674	.1548	.1116	24.76	3.24	100	100	91
25	.2317	.2023	.1719	31.53	1.99	100	100	100
30	.2642	.2696	.2401	37.41	1.37	100	99	100

[1] The pollinations were controlled as to time by bagging the ears and pollinating them by hand at a specific time

As in the former seasons, the seeds cut from the cob did not germinate as well as those allowed to dry on the cob. When the ear was partly dried on the stalk some grains germinated even when the plant was harvested the second day after pollination. Only a few grains harvested at the early stages germinated, and the results were irregular. On early harvested ears most of the grains cease development, but a very few continue to develop as long as they can receive nutrients from the cob. Culpepper and Magoon (4) noted that the grains upon ears of mutilated plants differ greatly in their chemical composition. Some grains continued their development almost normally, while others ceased to develop soon after the mutilating treatment cut off the food supply to the ear. This variation in the development of individual grains doubtless is responsible for at least some of the irregularities in the results. There were apparently differences in ears and a possible difference in strains, but it was obvious that at least many of the grains received a certain amount of nutrients from the cob after harvest.

In the third year of the work 11 additional varieties were employed in the germination studies. The methods were the same as for Stowell Evergreen and Golden Bantam, but as a consequence of differences in rate of development the harvesting of all the varieties at one particular age after pollination could not be made on the same day. They were harvested in August, there being not more than 5 days' difference in the dates on which material of a given age was harvested for the different varieties. The conditions were fairly uniform during the period and were characterized by high temperatures, which resulted in a rapid development of the grains, and consequently germination was obtained in most cases in very young material.

Germination was first obtained in the 7- to 8-day samples with the varieties Mammoth Late, Howling Mob, Money Maker, Country Gentleman, and Bantam Evergreen, and in 9- to 11-day samples of Sweet Orange, Stabler Early, Sunnybrook, Long Island Beauty, and Golden Sunrise. The earliest age of harvest of seed at which it will survive planting in the field was not determined. Seeds harvested 19 to 20 days after pollination approached 100 percent germinating power.

Apparently, only rarely can germination be expected of seeds from ears harvested less than 10 days after pollination, and such seedlings are so weak that they cannot develop into normal plants. As conditions are made more favorable for the survival of the embryo, the greater become the chances that it can develop into a normal plant.

The 3 years' results suggested that it would be possible to harvest sweet corn when it is 20 days of age from date of flowering, or at a time when it has maximum table quality, to make chemical analyses and other tests for the identification of desirable characteristics upon a portion of the ear and to dry the remainder and preserve it for subsequent planting. If the vigor of the plants from the immature seeds were not too much reduced, it would be feasible to make selections in this way with a knowledge of the particular qualities of each plant under consideration at the period of best table quality.

In cooperation with D. N. Shoemaker, the writers made more than 200 selections of self-pollinated ears of the varieties Golden Bantam and Stowell Evergreen. These were harvested 20 days after pollination, and half of each ear was used in making chemical analyses, tests for tenderness, and other tests, and the remainder was dried for planting the following year. The seeds of each of these immature ears were planted in the field and grown in the ordinary way without any special culture treatment. As would be expected, there was lowered vigor due to the effect of self-pollination, and this depressed further the decreased vitality resulting from the lessened reserves of food materials in the grain, thus making the young plants much less able to survive unfavorable conditions. In most cases, however, the seed from individual ears produced a crop of plants that was entirely satisfactory for the purpose of further selection. In many progenies the growth was practically as satisfactory as that obtained with corn harvested at complete maturity, while in others very few plants survived to maturity. The Stowell Evergreen proved somewhat more satisfactory than the Golden Bantam.

EFFECT OF DELAYED HARVEST ON GERMINATION OF SEED

In the third and last year of the work, open-pollinated ears were harvested at 5-day intervals from 30 to 70 days from the date of the first appearance of the silks. The results of the germination tests upon these are given in table 3.

It is noted that beyond the 45-day stage the percentage of grains germinating decreased in both varieties. At 70 days from silking, only 57 percent of Golden Bantam and 52 percent of Stowell Evergreen germinated. This decrease in the percentage of germination was obviously due to the attacks of micro-organisms that found the ear a favorable place for growth.

Because of the very warm, often rainy and humid conditions prevailing in the middle of the summer in the Middle and South Atlantic States, the ears often do not dry out rapidly enough in the husk in the field to prevent decay. The corn-earworm often causes injury to the tip of the ear that may allow the entrance of rain water, and this, with the injury to the tissues, favors the development of the decay organisms. If seed is to be saved in this section of the country, it is generally necessary to dry the ears artificially.

From the results of these tests the most favorable time of harvest appears to be between the 35- and 45-day stages, or at a time when the grains have reached a dry-matter content of 35 to 50 percent. Excellent germination is obtained at 30 days of age, but the grains are generally so soft as to be easily injured by rough handling at this stage. It is ordinarily best to harvest the corn before the plant is entirely dead, but the ears should be practically mature. The plants usually have the appearance of field corn at about the time it is usually cut and shocked. Many of the leaves are beginning to turn brown and die, but the stalk and part of the leaves are more or less green.

TABLE 3.—*Effect of delayed harvest on the dry-matter content, the air-dry weight, and the germination of the sweet corn varieties Golden Bantam and Stowell Evergreen*

Age from date of pollination (days)	Golden Bantam			Stowell Evergreen		
	Dry matter	Air-dry weight per seed	Germination	Dry matter	Air-dry weight per seed	Germination
	Percent	*Gram*	*Percent*	*Percent*	*Gram*	*Percent*
30	44.22	0.209	98	37.41	0.269	100
35	45.14	.198	100	36.32	.234	99
40	56.93	.214	98	51.60	.256	98
45	53.40	.198	100	50.72	.244	96
50	62.81	.218	95	58.74	.264	97
55	69.14	.201	91	65.29	.239	85
60	65.40	.220	80	76.13	.271	76
65	74.10	.236	72	64.26	.242	66
70	70.80	.216	57	69.34	.231	52

SUMMARY

With material properly dried, an almost perfect germination of the grains of sweet corn was obtained with ears harvested long before the corn reached complete maturity. In the very early stages the power of germination varied greatly with the age or maturity of the material, and there appeared to be no specific age or moisture content below which all the grains abruptly ceased to germinate. Below a certain age germination was progressively poorer the earlier harvesting was done. With ears harvested very early the grains germinated better when allowed to dry on the cob than when removed from the cob before drying. If the ear was allowed to remain on the cut stalk during the drying process, germination was still better. Apparently at least small quantities of nutrients are derived from the cob and stalk after harvest if the drying is not too rapid. It appears that on ears harvested very young and slowly brought to an air-dry condition certain grains develop much more fully than others and hence germinate while the others fail.

Results have been obtained indicating that the ears of sweet corn may be harvested at the proper stage of maturity for table use, tests made upon part of the ear for desirable table characteristics, and the remainder dried and preserved for subsequent planting. In this way selections of individual plants may be made at the time when the grains are at the proper edible stage rather than from the mature material. It was found possible to continue this treatment through three successive generations, thus giving an opportunity for the segregation of desirable characters not apparent in the mature grain.

When the ears of sweet corn are permitted to remain upon the stalk in the field in the vicinity of Washington, D. C., until fully air-dry, the grains may germinate very poorly, owing to the fact that microorganisms attack the material under the warm, humid conditions of this locality. For the production of good seed artificial drying is generally necessary. For this purpose the material should be harvested 35 to 45 days from date of flowering, or when the dry-matter content of the grain has reached 35 to 50 percent.

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THE CAUSES OF STILLBIRTH IN SWINE AND AN ATTEMPT TO CONTROL IT [1]

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INTRODUCTION

The death of young pigs just before or during birth is a common phenomenon, yet little attention has been paid to it. It is difficult to obtain reliable data on the extent to which it occurs, since information collected from breeders is usually incomplete. They do not bother, in many cases, to record all the dead pigs. Another source of error of the opposite sort is the listing of mummified embryos born with the litter. Most reports in the literature concerning still-born pigs are incidental to other work and usually it has been necessary for the writers to calculate the percentage incidence for comparison with their data. A summary of the data found is given in table 1. The normal mortality for the United States lies between 5 and 8 percent. The numbers shown in table 1 are fairly uniform except the percentage for New Zealand, which is high, and that for Slovakia, which is low.

King (*4*)[2] gives the natal mortality in rats of the Wistar Institute strain as 1.31 percent for 31,670 rats born. She believes that not more than 8 percent of still-born rats were not recorded, so that "it would appear that the normal birth mortality in the colony, under existing conditions of environment and of nutrition, was not greater than 2 percent." She summarized data on human births collected by the United States Children's Bureau in selected cities of the United States, and found 4.4 percent still-born in 5,456 births, a figure more comparable to the swine figures than is her percentage for rats. Probably mortality would be higher in rat colonies not so meticulously cared for as those of the Wistar Institute.

Table 1.—*The incidence of still-born pigs as reported by different authorities*

Authority	Pigs born	Dead at birth	Source of data and remarks
	Number	*Percent*	
Carmichael and Rice (*2*)	5,840	8.1	Ill. Agr. Expt. Sta. data, includes immature pigs
McKenzie (*7*)	[1] 2,011	[1] 8.2	Mo. Agr. Expt. Sta. data
Aubel, Hughes, and Lienhardt (*1*)	[1] 161	8.1	Kans. Agr. Expt. Sta. data. Sows fed basal ration
Aubel, Hughes, and Lienhardt (*1*)	169	[1] 2.4	Kans. Agr. Expt. Sta. data. Sows fed basal ration and wheat-germ meal
Lush, Hetzer, and Culberston (*6*)	3,639	[1] 5.8	Iowa Agr. Expt. Sta. data
McPhee and Zeller (*9*)	8,991	[1] 5.5	Beltsville Research Center, U. S. Department of Agriculture data
Kříženecký (*5*)	31,686	[1] 3.2	Controlled Pig-Raisers' data, Slovakia
McMeekan (*8*)	4,190	9.6	Pig Recording Club, New Zealand
Russell [2]	7,749	7.5	Data from 5 United States experiment stations
This paper	1,882	6.6	Data from Cornell University herd, Ithaca, N. Y.

[1] Calculated for this table.

[2] Russell, E. Z. THE RELATION OF BIRTH WEIGHT TO GAINS OF PIGS. U. S. Bur. Anim. Indus., Anim. Husb. Div. Mim. Paper, 3 pp. 1933.

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[1] Received for publication December 7, 1940.

[2] Italic numbers in parentheses refer to Literature Cited, p. 369.

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The work of Aubel, Hughes, and Lienhardt (*1*) suggests that mortality might be greatly reduced by the addition of wheat-germ meal to the ration of the sow, but in view of the composition of the basal ration, it is difficult to see in what way this could have improved it. The most thorough investigation of the causes, or rather the relationships, of stillbirth in pigs, is that of McPhee and Zeller (*9*). They found that stillbirth was related to litter size, as the smallest (1 to 2) and the largest (15 and above) litters had the greatest mortality at birth. To explain this they suggest that the smaller litters suffer most because they may be but the surviving remnants of litters that were larger at conception. The greater incidence of stillbirth in the largest litters is put down to the heavy physiological demands on the mother. They list the following as probable factors which influence birth mortality:

1. Errors in prenatal development
2. Nutrition, age, and care of the mother
3. Pathology
4. Lethal factors
5. Inherited endocrine disturbances

This classification is good, though it is doubtful whether a distinction can be drawn between factors 4 and 5. The list might be improved by adding one new class, accidental death, and a second, delayed birth, which might be a subdivision of class 2.

In the work herein recorded the writers have attempted to contribute to the knowledge of stillbirth in pigs through the collection of accurate records over a number of years and by autopsy of still-born pigs. When it seemed evident that delayed birth is a very important factor, an attempt was made with apparent success to reduce the mortality by the injection of pituitrin after the birth of the first pig in each litter. But the significance of the results obtained by this method now seems to be somewhat doubtful.

EXPERIMENTAL METHODS

Records were kept from the spring of 1930 of all pigs born alive or dead in the Cornell University herd. Whenever possible the order of birth and the birth weights were also obtained. The dead pigs were preserved in jars containing 5 percent formalin until about 100 had been saved. Mummified embryos were not counted or saved.

The university herd consists of purebred Berkshires, Chester Whites, and Duroc-Jerseys, with a very few crossbreds. The feeding and management have been kept fairly constant since 1930, but naturally there have been seasonal changes as well as changes of herdsman. Matings are made to get two litters yearly from the sows, in early spring and early fall. At the beginning of the period under discussion, the proportion of aged pigs was high, but by 1935, the end of the collection period, it had become the custom to keep the sows for a much shorter time so that the proportion of first litter sows was greater.

INCIDENCE OF STILLBIRTHS

During the period under discussion, 1930 to 1935, inclusive, the total number of pigs born was 1,882, of which 125 were dead. This is 6.6 percent, a proportion that compares favorably with the other figures from the United States. There was little breed difference, but it is noticeable that the incidence of still-born pigs is in the order of average litter size (table 2). This may be important as it is shown in Table 3 that litter size has an influence on the percentage of pigs born dead.

TABLE 2.—*Stillbirths in pigs as related to breed and average litter size*

Breed	Average litter size	Still-born pigs
	Number	*Percent*
Duroc-Jersey	10.6±.31	7.2
Berkshire	10.5±.30	6.7
Chester White	10.1±.25	6.0

The size of the litter seems to have had some effect on the percentage of stillbirths (table 3). The figures for small litters are too scanty to give reliable information, but it is evident that with very large litters the birth mortality tended to increase. This is in accord with the results of other workers. Carmichael and Rice (*2*) found that the trend was high with small and large litters. McPhee and Zeller (*9*) observed the same trend. However, the writers question their suggestion that the increased mortality in large litters is due to an increased physiological drain on the mother, for the evidence in this work indicates that the increased duration of the birth process is an important factor.

TABLE 3.—*Stillbirths in pigs as related to litter size*

Litter size	Litters	Dead pigs	Still born pigs	Litter size	Litters	Dead pigs	Still-born pigs
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
1 to 5	10	0	0	11	21	13	5.6
6	3	1	5.6	12	20	18	7.5
7	11	2	2.6	13	13	17	10.1
8	20	8	5.0	14	18	21	8.3
9	19	1	.6	15 to 20	16	31	12.2
10	26	8	3.1				

The age of the sow is another possible factor. The figures in table 4 show a definite trend toward an increase in birth mortality with the increase in the age of the sows. Křiženecký's (*5*) data show a similar trend from the first litter to the seventh, but afterwards there is a somewhat erratic decrease. Russell's[3] data show a fairly steady increase with age as definite as those of the present writers. McMeekan's (*8*) figures are more erratic but show the same trend.

TABLE 4.—*Stillbirths in pigs as related to age of the sow at time of delivery*

Age of sow	Litters	Still born pigs
	Number	*Percent*
9 months to 1 year, 3 months	41	2.7
1 year, 3 months to 1 year, 9 months	38	5.2
1 year, 9 months to 2 years, 3 months	38	3.5
2 years, 3 months to 2 years, 9 months	23	9.6
2 years, 9 months to 3 years, 3 months	14	9.8
Above 3 years, 3 months	20	14.1

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[3] RUSSELL, E. Z. AGE AS A FACTOR IN BROOD SOW PERFORMANCE. U. S. Bur. Anim. Indus., Anim. Husb. Div. Mim. Paper, 4 pp. [n. d.]

There seems to be a seasonal influence also, as the mortality in 92 litters farrowed in the spring was 9.2 percent and in 88 litters farrowed in the fall, 4.9 percent. McMeekan's (*8*) pigs farrowed over a much longer period than did those of the writers. Taking comparable periods, his fall farrowings were the higher, with 12.5 percent mortality, and his spring farrowings the lower, with 5.0 percent mortality. The winters in the Manawatu-Oroua district of New Zealand in which these pigs lived are very mild with rare frosts, and there is far less difference between seasons than at Ithaca, N. Y., where the writers' pigs are kept. Evidently, if the Ithaca and Manawatu-Oroua data are representative, the factors at work which influence the seasonal incidence are totally different in the two localities. King's (*4*) rat data give a higher incidence of stillbirths in the fall (3.04 percent) than in the spring (1.78 percent). These figures for rats are in accord with those from New Zealand for pigs. Perhaps the Ithaca results reflect the fact that the sows had several months of pasture feeding before the fall farrowing and only 1 month before the spring farrowing. Support for this view is gained from the fact that after the close of the work described in this paper, many sows were not given summer pasture and the incidence of stillbirths was high in the spring farrowing. Many of the dead pigs showed signs of thyroid (iodine) deficiency. Superficial edema, thickening of the fore limbs, and enlarged thryoids were observed. Ithaca is on the edge of the Great Lakes iodine-deficiency area.

The sex ratio of all the pigs born was 52.5 percent males, and that of the dead pigs 51.2 percent males. This may represent a very slight tendency for females to die more readily than males. Carmichael and Rice (*2*) found a sex ratio of 56 percent males in 402 still-born pigs. McPhee and Zeller (*9*) found that the sex ratio of dead pigs was 52.9 percent males (497 pigs) as compared with 52 2 percent males for all pigs. Evidently there is little or no tendency for mortality to be greater in one sex than in the other. King (*4*) however, found a very great excess of male stillbirths in her rats and also in her human data.

POSSIBLE CAUSES OF STILLBIRTH

There is no doubt that the tendency is for still-born pigs to be the more immature pigs in the litter, but this tendency is not complete, for dead pigs may be found in any weight class. The average weight of all pigs born was 2.6±0.02 pounds, while that of the dead pigs was 2.1±0.07 pounds, a difference of 0.5 pound. The coefficient of variation for all pigs born was 26; for pigs born dead it was 38. Lush, Hetzer, and Culbertson's (*6*) equivalent figures are 2.7 and 2.2 pounds, with approximate (calculated) coefficients of variation of 20 and 30 respectively. The weights of pigs born dead are, therefore, much more variable than those of all pigs born.

The still-born pigs saved were weighed, measured, and dissected. Weights were taken for the liver, stomach and intestines (empty), kidneys, lungs, brain, and heart. The averages with their constants are given in table 5. No similar figures for normal swine are available for comparison but the rank of the organs in degree of variability follows very closely that for normal newborn rats given by Jackson. (See table 5.) The variability in individual organs is naturally much greater, since there is far greater variability in body weight. The heart shows this more than any other organ.

TABLE 5.—*Average weights of organs in still-born pigs as compared with similar data for newborn rats*

Organ	Mean	Probable error	Standard deviation	Coefficient of variation	Jackson's (5) coefficient of variation [1] for newborn rats
Total weight	947 gm.	±23	358	38	[1] 12
Crown-rump length	29.2 cm	±.3	4.4	15	
Brain	29.2 gm	±.3	4.2	14	12
Heart	9.6 gm	±.2	3.2	33	18
Kidneys	7.6 gm	±.2	2.5	33	24
Liver	21.4 gm	±.5	8.3	39	22
Lungs	22.3 gm.	±.6	9.1	41	23
Stomach and intestines (empty)	44.3 gm.	±1.2	19.0	43	[2] 38

[1] Approximate

[2] With contents.

An attempt was made to decide for each of the 127 pigs dissected the probable cause of death. This could be no better than guesswork in many cases as it is difficult to decide to what extent extreme variation in the size of an organ is lethal. Where the difference in the weight of one organ was great as compared with the rest of the organs and with the body weight, the fact was noted and was given as the probable cause of death. Many of the pigs undoubtedly failed to live because of immaturity, but it is difficult to distinguish in all cases between smallness and immaturity of a lethal nature. It was decided arbitrarily that a pig smaller than 375 gm. would not be likely to live, but this is probably a very conservative estimate. There seems to be no reason to assume that excessive size would be lethal except as it affects the mechanics of birth.

When any organs were doubtful in appearance or showed gross lesions, they were sectioned and studied histologically. Paraffin embedding with haematoxylin and eosin staining was employed. Potassium ferrocyanide was used for the detection of iron in the liver and spleen.

The assigned reasons for failure of these pigs to live are as follows:

Immaturity and smallness	8
Disproportionate organ weights	18
Pathology	19
Endocrine disturbances and edema	19
Deformities of skeleton	5
Accidental death (e. g., birth injuries, crushing before herdsman saw pig)	13
No obvious reason for death	45
Total	127

The group designated "Disproportionate organ weights" may be subdivided as follows: Large heart, 5; large heart and kidneys, 2; large heart and brain, 1; large brain, 4; small brain, 5; small liver, 1. It is difficult to decide what effect an enlarged heart would have upon the viability of the pig, but the hearts in these cases were extremely disproportionate as compared with the average. This is reflected, of course, in the high coefficient of variation already noted. Brain disproportion is probably a much more serious fault.

Under the heading "Pathology" two main types of lesion were noted: (1) White spots on the surface of the liver, caused by an extensive invasion of white blood cells (half of one large litter was born dead, with these lesions in each pig), and (2) black spots on the spleen or on the spleen and liver, caused by heavy deposits of iron compounds. Other lesions listed were kidney and heart small, and necrotic calcified masses on left cornea; blood in thorax, lungs pitted and edematous, 2 cases; coagulated serous fluid in thorax, 2 cases; emphysema of lungs; liver atrophied, coagulated serous fluid in thorax.

It is realized that these descriptions are very incomplete and that the nature of the preservation of the pigs left much to be desired for this type of work. The descriptions are meant to indicate merely that pathology was present in these cases. Edema, when it occurred alone, was taken as indicative of thyroid deficiency and was classed under the heading of endocrine disturbances.

No swine abortion (*Brucella suis*) infection has been found in the herd during the course of this work.

The classification "Endocrine disturbances and edema" is somewhat vague. It was made because in a few cases edema was found to be associated with fairly obvious abnormalities in the size of the thyroid or thymus gland, but in most instances it was not so accompanied. Details and number of cases in this class are as follows:

Thymus large, edema, 2.
Thyroid large, edema, 2. In one of these the brain was small. There was very little hair. The right kidney was small and necrotic; the left kidney was hypertrophied so that the weight of the two together was close to the average for these organs.
Thymus large, neck rigid, 1.
Thymus large, 1.
Thyroid and spleen large, histology normal, 1.
General edema, 11.
Umbilical cord large and edematous, 1.

Skeletal and other deformities were few. They were as follows: Thickening of lower jaw, 2 cases (in one of these general edema was also present); hind legs drawn back, brain and alimentary canal small, 1; extreme hernia, 1; cleft palate, brain abnormally small, alae of nose not united, 1.

Accidental deaths were divided as follows: Fractured or compressed skull, 4; intracranial hemorrhage, 2; extensive bruises, 3; excessive hemorrhage from umbilical cord, 1; umbilical cord tight around body, 1; swallowed fetal membranes, 1; ruptured jugular vein, 1.

In all, about two-thirds of the deaths were provisionally accounted for, but it is obviously impossible to decide what proportion of these should be charged to heredity and what to disease processes. The small number of structural anomalies is surprising.

After a number of the pigs had been dissected, it was realized that there was considerable difference in the appearance of the lungs when they were sliced. Some had obviously been inflated, but many had not. The lungs of the last 105 pigs were sectioned and examined histologically. The results are striking: Alveoli collapsed, 35; alveoli partly open, 46; alveoli wide open, 24.

Many of the pigs die after they have breathed, many others are able partly to inflate their lungs, but die before they are able fully to inflate them. Others are dead before any attempt at inflation can be made. This led to the belief that many of the pigs were suffocated within the uterus or vagina. This conclusion was strengthened by the fact that stillbirth was known to occur more frequently when the farrowing was unduly prolonged. The question arose whether the order of birth was also a factor. In several farrowings the exact order of birth had been recorded. These records showed definitely that the great majority of the still-born pigs were born late in the litter. Figure 1 illustrates this. The vertical line represents the division of litters into the first and second half farrowed. Still-born pigs are represented by an X. Twenty-six litters in all are charted. Nine pigs were born dead in the first half of the pigs farrowed, and 45 in the second half.

			1	2	3	4	5	6	X	8	9	10	11		
	1	2	3	4	5	6	7	8	9	10	11	X	X	14	15
			1	X	3	4	5	6	X	8	9	10			
		1	2	3	4	5	6	7	8	9	10	11	X	13	
					1	2	X	4	5	6	7				
				1	2	3	4	5	6	7	X				
		1	2	3	4	5	X	7	8	9	10	11	12		
								X	X	X	*				
1	2	3	4	5	6	7	8	9	10	11	X	X	14	X	X
		1	2	3	4	X	6	X	X	9	X	X	X	X	
		1	2	3	4	5	6	7	8	9	10	X	X		
	1	2	3	4	X	6	7	8	9	10	11	12	13	14	X
			1	2	3	4	5	6	7	8	9	10	X		
	1	2	3	4	5	6	7	8	9	10	11	12	13	X	X
		1	2	3	4	5	6	7	8	9	X	11	12	13	
1	2	3	4	5	6	7	8	9	10	11	X	X	X	X	X
	1	2	3	4	5	6	7	8	X	10	11	X	X	X	
1	2	3	4	5	6	X	8	9	10	11	12	13	14	15	16
		1	2	3	4	5	6	7	8	9	X	11	12		
		1	2	3	4	5	6	X	8	9	10	X	12		
	X	2	3	4	5	6	7	8	9	10	11	12	13	14	
			1	2	3	4	5	6	7	8	9	X	11		
			1	2	3	4	X	X	X	8	9	10	X		
		1	2	3	4	5	X	7	8	9	10	11	12		
				1	2	3	4	5	6	7	X	X			
	1	2	3	4	5	6	7	8	9	10	11	12	13	X	
9 DEAD PIGS								45 DEAD PIGS							

FIGURE 1.—Pigs still-born in the first and second half of the litters farrowed. The litters are recorded horizontally in the order of birth of the pigs with the dividing line at the center of the litter. Still-born pigs are indicated by X; asterisk (*) indicates 3 dead pigs born in the second half of a litter of 13.

AN ATTEMPT TO REDUCE THE INCIDENCE OF STILLBIRTHS

In the light of the foregoing results it seemed possible that mortality might be reduced by hastening birth. Accordingly, for the next five farrowing seasons—1936 to spring, 1938 2 cc. of pituitrin, U. S. P. IX at first and later 1 cc. of pituitrin U. S. P. XI, was injected into each sow immediately after the farrowing of the first pig or as soon thereafter as possible. The injection was made subcutaneously in the soft part of the neck, 2 or 3 inches behind the ear, the skin having first been swabbed with alcoholic iodine or alcoholic mercuric chloride solution. During the period of the experiment 1,148 pigs were farrowed, and 33 of these were dead. This was a mortality of 2.9 percent. The average weight of the dead pigs was 2.1 pounds, the same as previously. These results seemed to justify the conclusion that the injection of pituitrin had markedly cut down the mortality, as there was a drop from 6.6 percent to 2.9 percent.

In order to establish a check on this result, during the next five farrowing seasons (fall, 1938 through 1940) insofar as possible alternate sows were injected. Of 352 pigs born to injected sows, 15 or 4.3 percent were still-born. Five of these pigs were born in one litter after a long delay in farrowing. Of 545 pigs born to sows that were not injected, 13 or 2.4 percent, were still-born. The difference in the number of pigs farrowed in the 2 groups is due not to a difference in litter size, but to the fact that more sows were left uninjected. Even if the 5 dead pigs in 1 litter were omitted, the injections would not have lowered the mortality below that in the noninjected sows. Furthermore, the current rate of mortality is exceptionally low, 2.9 percent, for all pigs during the 5 years of injections, a figure below that of any comparable series in the literature. No changes in management of the sows had been made that would appear to account for this decline. The writers are at a loss to explain it and they hope that others will repeat this work in order that further data may be obtained on the effects of pituitrin injections. The lower average age of sows in the second 5 years may account for some of the decline, but only for a small part.

SUMMARY AND CONCLUSIONS

The percentage of still-born pigs in the Cornell University herd over a period of 5 years was 6.6.

Large litters and advancing age of the sow tend to increase the birth mortality. Spring farrowings had nearly twice the mortality of fall farrowings. The mortality was no greater in one sex than in the other.

The average weight of still-born pigs was 2.1 pounds, as compared with 2.6 pounds for all pigs farrowed. The weight of still-born pigs was also more variable than that of all pigs farrowed.

As far as possible, the cause of death was ascertained. Disproportionate organ weights and pathological conditions seemed to be the principal contributory factors.

A considerable proportion of the pigs had made abortive attempts to breathe and had been smothered before birth. Most of the dead pigs were born late in the farrowing.

An attempt by the injection of pituitrin to hasten birth and thus reduce the mortality seemed to be successful at first, but subsequent work has thrown doubt on its value.

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COMPARATIVE INJURY BY THE EUROPEAN CORN BORER TO OPEN-POLLINATED AND HYBRID FIELD CORN [1]

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INTRODUCTION

Hybrid field corn (*Zea mays* L.) adapted to local conditions has been found superior to the common open-pollinated varieties of corn with respect to resistance or tolerance to some diseases and insects, the number of barren or earless plants, the degree of stalk breakage, and the yield of marketable grain. The possibility of its superiority with respect to tolerance to infestation by the European corn borer (*Pyrausta nubilalis* (Hbn.)) prompted the inclusion of several open-pollinated varieties among test plantings of hybrids in northwestern Ohio during the years 1929–33. The locally adapted Wooster strain of the open-pollinated Clarage variety was also grown by itself for comparison with the open-pollinated varieties as a group, not all of which were locally adapted. The determination of the comparative amount of injury to the quality and yield of the ears and also of breakage of the stalks was the primary purpose of this study.

A study of the injury to the quality of the ears should include, in addition to their marketability, the feeding value of the grain. This phase of the problem has been investigated by the Bureau of Animal Industry, United States Department of Agriculture, and the Chief of that Bureau made the following statement in his annual report for 1933: "Analyses of the separate parts of plants infested by corn borers show that the leaf, stalk, grain, cob, and husk were not markedly changed in composition. Reduced production in corn-borer-infested areas, therefore, can be measured chiefly on the basis of reduced yield." Accordingly, no additional data on grain analysis are presented in this paper.

STRAINS OF CORN USED

The group of open-pollinated varieties was made up, in 1930, of Red Cob Ensilage, Longfellow Flint, and the European varieties Telbisz Early Dent, Pferdezahn, and Bankuti Dent; in 1931, of Golden King, Krug, and Woodworth's Reconstituted High Yield and Reconstituted Reid; and in 1933, of Johnson County White, Smoky Dent, Clarage, Canterbury, and Doubet. There were 22 hybrids in the group tested in 1930, 15 of which were single crosses, 4 were three-way crosses, 2 were double crosses, and 1 was a top cross; of 20 hybrids tested in 1931, 18 were single crosses and 2 were double

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[2] The authors gratefully acknowledge material aid and advice received from J R Holbert, formerly of the Bureau of Plant Industry, J S Cutler and M T Meyers, formerly of the Ohio Agricultural Experiment Station and the following workers in the Bureau of Entomology and Plant Quarantine, U S Department of Agriculture W H Larrimer (now with the Forest Service), D J Caffrey, W A Baker, and Morris Schlosberg The tests at Holgate, Ohio, during 1932 and 1933 were conducted on ground provided by the Ohio Agricultural Experiment Station

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crosses; and of 7 hybrids tested in 1933, 4 were single crosses, 2 were three-way crosses, and 1 was a double cross.

EFFECT OF BORER POPULATION ON THE QUALITY AND YIELD OF THE EARS

COLLECTION OF DATA AND STATISTICAL ANALYSES

The various strains of corn were subjected to different known levels of borer population. These strains were randomized within each of several replications. Each plot of a given strain was divided into as many equal subplots as there were levels of borer population in the experiment. One of the subplots was subjected to natural infestation only, whereas each of the others was, in addition, infested by hand with a definite number of egg masses per plant to produce the desired level of borer population. The eggs were produced in the laboratory.[3]

Some strains matured earlier than others, and the ears of these strains were a special attraction to blackbirds. Therefore, to avoid errors in comparing yields, all the ears were tightly covered with paper bags to protect them from the birds.

On an average 24 plants from each level of infestation of each strain were dissected after the middle of August to determine the mean number of borers per plant before many of the full-fed larvae had migrated away from the plants.

At harvest time record was made by subplots of the total number of productive plants, the number of earless plants, the number of plants bearing two ears, the number and weight of all the ears harvested, and the number and weight of the unmarketable ears. Plants maturing less than six kernels of grain were recorded as earless. Unmarketable ears were the chaffy, rotted, or moldy ears and nubbins so small that they would not ordinarily be harvested. Ears of sound grain, even though small, and ears with only a few of the kernels moldy were classified as marketable.

From the total number of plants in all the subplots of each level of infestation, the percentage that was earless and the percentage and the weight of ears that were marketable were calculated. These values were placed on an acre basis for a stand of 10,000 plants, thus permitting easy conversion of the numbers of plants per acre into percentages, and vice versa. The actual stands of 10 plantings of Clarage and 7 plantings of open-pollinated groups and of hybrids averaged 10,100 and 10,383 plants in 3,556 hills per acre, respectively. The yield was calculated in bushels of 56 pounds each of shelled corn on the basis of a moisture content of 15.5 percent.

The data from the 1930 planting of open-pollinated varieties, which averaged one borer per plant, are given as an example of the conversion of the data to an acre basis. On an average 5.5 percent of the plants, or 550 plants per acre, were earless. Therefore, 9,450 plants per acre were productive. Of the ears harvested, 93.0 percent were classified as marketable. On the assumption that each ear represented a productive plant, 8,789 plants per acre bore marketable ears and 661 plants per acre bore unmarketable ears. Of the ears harvested 98.6 percent by weight were classed as marketable. Since the estimated yield of the full stand of plants was 57.4 bushels per acre, the estimated marketable yield was 56.6 bushels per acre.

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Finally, the simple regressions of the number of earless plants per acre, the number of plants bearing marketable ears, the total yield, and the marketable yield on the level of borer population were calculated. Per-acre estimates were then made of the measurable quantities in the absence of borers, which in this paper are called the normal values. The results of the statistical analyses of all data are given in table 1.

As indicated in the example given above, one ear at harvest was assumed to represent one productive plant. Although the number of productive plants per plot was recorded, the identity of the individual ears from two-ear plants was lost when they were classified as marketable and unmarketable, and no record was obtained of the number of plants for each class of ears harvested. It was not possible, therefore, to determine the number of plants per acre bearing marketable ears. Two-ear plants, however, were few in number. During the period of the experiment the average percentage of two-ear plants of Clarage ranged from 0.4 to 2.4, with a general average of 0.9, and of the open-pollinated and hybrid groups from 0.5 to 4.6, with a general average of 2.1. The discrepancy introduced into the calculations by assuming an ear to represent one productive plant may therefore be considered as of no material importance.

The standard errors of the regression coefficients were calculated by Fisher's formula.[4] The variances of the different estimates in the absence of borers were calculated by a modification of the formula given by Ezekiel,[5]

$$e^2_{y'} = \sigma^2_{M_y} + (\sigma_{b_{yx}} M_x)^2$$

where $\sigma^2_{M_y}$ is the variance of the mean value of the factor of all borer levels, $\sigma_{b_{yx}}$ is the standard error of the regression coefficient, and M_x is the mean borer population of all levels.

The standard errors of the averages of all the plantings were calculated by the formula for the standard error of a general mean.

The number of degrees of freedom given in table 1 ranges from 1 to 4, indicating that the field data were obtained from three to six levels of borer population.

In estimating the effect of the infestation on the various factors, averages of all the plantings were used as a basis for comparison. Any differences between corresponding values of the Clarage and the open-pollinated groups may have been due to differences in variety, soil, planting date, or locality, but the values for the open-pollinated varieties and hybrids are directly comparable.

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TABLE 1.—*Per-acre estimates of the number and yield of plants bearing marketable ears, of the number of earless plants, and of the yield of the entire stand in the absence of borers, together with the expected increase, or decrease, per acre for each increase of one borer per plant for the tested types of corn, Ohio, 1929–33*

CLARAGE

Date of planting	Locality	Per-acre estimates in absence of borer				Increase (+) or decrease (−) per acre for each increase of 1 borer per plant				Degrees of freedom
		Plants bearing marketable ears	Earless plants	Yield of plants bearing marketable ears	Yield of entire stand of plants	Plants bearing marketable ears	Earless plants	Yield of plants bearing marketable ears	Yield of entire stand of plants	
		Number	*Number*	*Bushels*	*Bushels*	*Number*	*Number*	*Bushels*	*Bushels*	*Number*
1929										
May 17	Toledo	6,722±238.7	325±230.2	38.2±1.11	49.8	−171±12.8	+73±13.8	−1.00±0.060	−1.00±0.039	4
1930										
May 7	Sandusky	8,287±230.4	663±159.9	52.7± .75	53.8	−183±57.2	+132±39.6	−2.63± .186	−2.52± .185	2
May 9	Huron	6,726± 90.8	1,999± 45.9	42.3± .76	43.5	−77±22.5	+82±11.3	−1.87± .189	−1.84± .141	2
1931										
May 6	Sandusky	8,793±180.8	517± 95.8	65.7±1.04	66.8	−120±15.6	+71± 8.3	−1.99± .090	−1.91± .084	3
May 7	Huron	6,546±186.0	2,210±161.1	41.6± .22	43.5	−193±22.1	+141±19.1	−2.02± .027	−1.96± .016	3
May 13	Toledo	8,247± 42.8	959± 29.1	51.5± .98	52.6	−123± 7.0	+58± 4.8	−1.79± .160	−1.67± .162	3
1932										
May 10	Toledo	8,324± 54.3	876±130.2	49.8± .82	50.8	−70± 7.7	+73±18.4	−1.45± .116	−1.45± .095	3
May 11	Maumee	8,297±305.1	711±238.9	43.7±1.52	45.5	−146±42.4	+92±33.3	−1.60± .210	−1.57± .221	3
	Holgate	7,863±275.5	931±108.1	54.5±2.89	56.8	−80±48.0	+88±18.9	−2.01± .503	−2.08± .470	3
May 17	Sandusky	9,290± 28.7	211± 43.6	84.0±1.42	84.9	−47± 4.5	+44± 6.7	−2.32± .218	−2.34± .218	3
Mean		7,910± 60.0	940± 45.2	52.4± .42	54.8	−121± 9.4	+85± 6.5	−1.87± .068	−1.83± .064	29
HYBRIDS										
1930										
May 8	Sandusky	9,690± 38.1	92± 37.2	73.9±0.26	74.3	−38± 9.4	+24± 9.2	−2.88±0.063	−2.88±0.063	1
1931										
May 5	do	9,306± 61.6	431± .6	86.6± .91	87.1	−16± 5.2	+2± .05	−1.63± .077	−1.58± .064	1
May 12	do	9,198±253.8	481±243.0	81.9± .06	82.7	−60±21.0	+37±20.1	−1.81± .005	−1.77± .048	1
May 19	do	9,076± 69.2	387± 64.4	77.9± .94	78.8	−99± 8.7	+71± 8.1	−2.39± .118	−2.34± .113	1
May 25	do	9,182± 87.2	458± 51.0	79.6± .62	80.2	−79±12.5	+40± 7.3	−2.57± .089	−2.48± .073	1

1933										
May 19	Toledo	9,898± 48.4	30± 18.5	96.4±4.92	98.5	−119± 5.5	+16± 2.1	−3.49± .558	−2.97± .553	1
June 2	do	9,578±124.1	23± 25.2	96.2±1.48	99.7	−16±23.2	+11± 4.7	−2.92± .277	−2.85± .533	1
Mean		9,418± 45.1	272± 37.3	84.6± .76	85.9	−61± 5.3	+29± 3.6	−2.53± .093	−2.41± .112	7

OPEN-POLLINATED GROUPS

1930										
May 8	Sandusky	8,981±340.2	430±302.3	60.9±0.62	61.7	−238±67.5	+162±60.0	−3.28±0.122	−3.23±0.093	1
1931										
May 5	do	8,319±403.9	1,001± 81.3	70.1± .93	71.2	−29±33.0	+8± 5.6	−1.40± .076	−1.31± .020	1
May 12	do	8,006±806.0	1,696±737.6	66.5±2.87	66.6	−113±76.7	+16±70.2	−2.14± .273	−1.84± .227	1
May 19	do	7,794± 56.0	966±508.2	55.4±5.32	56.7	−179± 6.9	+130±62.7	−1.97± .656	−1.73± .911	1
May 25	do	7,673±151.0	1,544± 71.0	55.9±4.54	56.9	−151±18.9	+45± 8.9	−1.96± .568	−1.69± .569	1
1933										
May 19	Toledo	9.026±133.9	71± 72.6	71.7±5.61	81.9	−169±13.2	+84± 6.6	−2.78± .553	−2.64± .228	1
June 2	do	9,230± 88.5	408±222.9	82.1±3.38	86.1	−161±13.5	+59±34.0	−3.61± .516	−3.18± .623	1
Mean		8,433±141.4	874±140.0	66.1±1.44	68.7	−149±15.8	+72±16.8	−2.45± .170	−2.23± .184	7

REDUCTION IN MARKETABLE YIELD

The comparative injury by the borer to the yield of the three groups of corn was studied from the standpoint of the reduction in marketable yield. Under infestations up to about five borers per plant there were no clear visual indications of reduction in yield, although broken stalks and signs of infestation were numerous. Under severe infestation the most noticeable indication of reduction in yield was the increase in the number of earless plants, but some infested plants bore only unmarketable nubbins or injured ears. From experimental evidence the ears of all infested plants appear to be more or less reduced in size, but in most fields, unless they are heavily infested, a general reduction in size would pass unnoticed. The borer reduces the marketable yield of corn plants, therefore, in three ways—(class 1) through an increase in the number of earless plants, (class 2) through an increase in the number of plants bearing unmarketable ears, and (class 3) through a reduction in the size of the ear on all plants bearing marketable ears.

To study the effect of these factors in reducing the marketable yield, it was desirable to put the loss in yield due to smaller ears on a plant basis for comparison with the other two factors. The yield obtained at a given level of infestation is usually compared with an estimated yield in the absence of borers. Hence the loss in yield due to smaller ears is expressed in terms of the number of plants that would produce an equivalent yield in the absence of borers. Expressing the loss in this manner is the same as regarding the loss that actually occurred on all the plants bearing marketable ears as being concentrated on a number of plants to the extent of rendering them earless. Since the yield harvested at a given level of infestation requires more plants than are required to produce this yield in the absence of borers, class 3 represents the excess plants that would be needed to compensate for the loss due to smaller ears.

The number of plants in class 3 was determined by first dividing the number of bushels of marketable corn per acre at a given infestation level by the normal yield in fractions of a bushel per plant bearing marketable corn to obtain the number of plants that should have produced an equivalent yield in the absence of borers, and then subtracting this number from the number of plants bearing marketable ears per acre at the given level of infestation.

The number of earless plants, the number of plants bearing marketable ears, and the number of bushels of marketable yield were calculated on a per-acre basis for a level of 12 borers per plant from the values in the absence of borers and the increase or decrease in these values per borer. The number of plants per acre bearing unmarketable ears was found by deducting the sum of the number of plants bearing marketable ears and the number of earless plants from 10,000. The number of plants in each of the three classes, in the absence of borers and at an infestation level of 12 borers per plant, is given in table 2. The difference between these two levels gives the increase in the number of plants necessary to compensate for the loss due to 12 borers per plant. The totals of the differences give the number of plants whose production in the absence of the borer would have equaled the total loss in marketable yield due to the borer.

Table 2.—*Classification of the loss to the various types of corn due to corn borer infestation at an average level of 12 borers per plant, Ohio, 1929–33*

CORN BORER ABSENT

Type of corn	Plants per acre			
	Earless plants (class 1)	Plants bearing unmarketable ears (class 2)	Additional plants required to compensate for loss due to smaller ears (class 3)	Total
Clarage	940	1,150	0	2,090
Open-pollinated group	874	693	0	1,567
Hybrids	272	310	0	582
INFESTATION LEVEL 12 BORERS PER PLANT				
Clarage	1,960	1,582	1,930	5,472
Open-pollinated group	1,738	1,617	1,963	5,318
Hybrids	620	694	2,652	3,906
INCREASE NECESSARY TO COMPENSATE FOR BORER ATTACK				
Clarage	1,020	432	1,930	3,382
Open-pollinated group	864	924	1,963	3,751
Hybrids	348	384	2,652	3,384

From the number of plants in each class given in table 2, figure 1 was constructed to present the data graphically. A horizontal line was

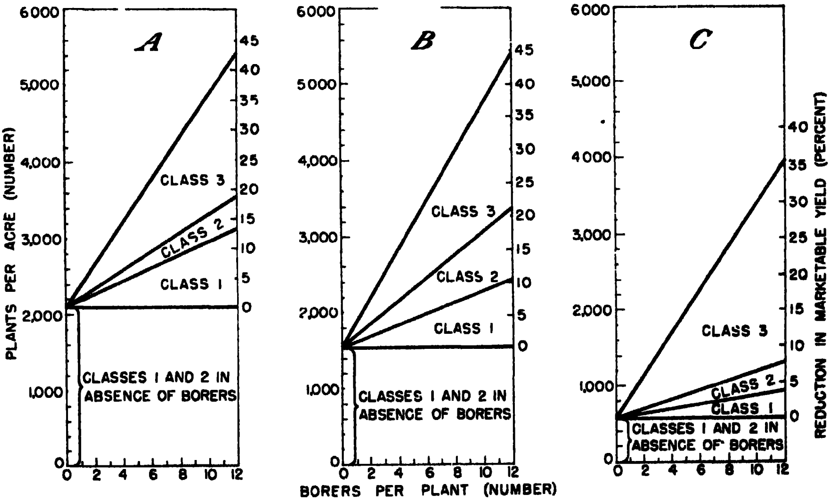

Figure 1. Reduction in marketable yield of corn, classified according to character of injury resulting from corn borer attack at different levels of infestation: *A*, Clarage; *B*, open-pollinated group; *C*, hybrids.

first drawn at the level of the total number of earless and unmarketable ear-bearing plants in the absence of borers. Then the total increase in the number of plants necessary to compensate for borer attack at the 12-borer level was plotted above the horizontal line.

These points were connected with the origin at the no-borer level by a straight line. At the right of each figure a supplementary scale gives the percentage reduction in marketable yield due to the borer. This scale was erected on the basis of the total increase in number of plants necessary to compensate for the attack of 12 borers per plant as a percentage of the number of plants bearing marketable ears in the absence of borers. For example, the total increase of 3,382 plants in the Clarage strain is 42.76 percent of 7,910, the number of plants bearing marketable ears in the absence of borers.

The size of the ears was reduced about the same percentage in all three groups, because the ears on all the plants bearing marketable ears were smaller and the total reduction in marketable yield due to the corn borer was equivalent in each case to the yield from about the same number of plants in the absence of the borer. These results were obtained even though half the tests on Clarage were conducted during seasons in which the hybrids and the open-pollinated group were not tested and all but two of the tests on Clarage were conducted at different localities.

In the absence of borers 7,910 plants bearing marketable ears of Clarage yielded 52.4 bushels per acre (table 1), or 0.006624 bushel per plant. Owing to increases in the number of earless plants and plants bearing unmarketable ears, the number of plants bearing marketable ears was reduced 121 plants per acre per borer per plant, so that in the presence of 12 borers per plant 6,458 plants bore marketable ears. In the absence of borers, these 6,458 plants would have yielded 42.8 bushels of marketable grain. Since the marketable yield was reduced 1.87 bushels per acre per borer per plant (table 1), the yield was 30.0 bushels in the presence of 12 borers. Hence the yield was reduced 12.8 bushels and the size of the ears 29.9 percent owing to smaller ears on all the plants bearing marketable ears.

By similar calculation it may be shown that the size of the ears of the hybrids and the open-pollinated varieties was reduced 30.5 and 29.5 percent, respectively.

Since the marketable yields of Clarage, the hybrids, and the open-pollinated varieties were reduced, respectively, 1.87, 2.53, and 2.45 bushels per acre per borer per plant, an average of 12 borers per plant would reduce the yield 22.4, 30.4, and 29.4 bushels per acre, respectively. In the absence of borers a plant bearing a marketable ear averaged 0.006624, 0.008983, and 0.007838 bushel for the respective groups. Therefore, the total reduction in marketable yield due to 12 borers per plant was equivalent to the yield in the absence of borers of 3,382 plants of Clarage, 3,384 plants of the hybrids, and 3,751 plants of the open-pollinated varieties. These totals, calculated by another method, are given in table 2.

RELATIVE BORER DAMAGE TO DIFFERENT STRAINS

From the foregoing data it appears that the total reduction in marketable yield per acre per borer per plant was equivalent to the normal yield of an average of about 292 hybrid or open-pollinated plants, and that the size of the marketable ears of both the hybrids and the open-pollinated varieties was reduced on an average about 2.50 percent per borer per plant.

Although the total reduction in marketable yield due to 12 borers per plant was equivalent to the normal yield of about the same number of hybrid and open-pollinated plants, the loss of the hybrids was equivalent to the normal yield of 3,384 plants, which is 35.93 percent of 9,418, the number of plants bearing marketable ears in the absence of borers, whereas the losses of Clarage and the open-pollinated group were equivalent to 42.76 and 44.48 percent, respectively, of the normal yield. The loss for the hybrids was 2.99 percent per borer per plant as compared with 3.56 percent for Clarage and 3.71 percent for the open-pollinated group, or an average of 3.64 percent. Hence the hybrids were more tolerant to the borer than the open-pollinated varieties in that a given number of borers caused about five-sixths as much loss of yield to the former as to the latter. This advantage was due to the difference in the rate of increase in the number of earless plants and those bearing unmarketable ears per acre per borer per plant. For the hybrids the increases were 29 and 32 plants, respectively, as compared with 72 and 77 for the open-pollinated plants on the same plantings. In correlation with these lower rates of increase, the hybrids in the absence of borers would have had a smaller number of earless plants and plants bearing unmarketable ears and a correspondingly larger number of plants bearing marketable ears. The hybrids would have had 9,418 plants per acre bearing marketable ears in the absence of borers as compared with 7,910 plants of Clarage and 8,433 plants of the open-pollinated group. All these differences between the hybrids and the open-pollinated group are shown in figure 1.

The greater tolerance of the hybrids over the open-pollinated group may be shown as due to a given number of borers causing a smaller number of plants to become earless or to bear unmarketable ears, and not to a higher level of performance in the absence of borers. If the hybrids had lost the yield from as many plants bearing marketable ears as was lost by Clarage and the open-pollinated group, the loss would have been 14.6 bushels per acre from an average of 1,620 plants. The hybrids, then, would have had only 7,798 plants bearing marketable ears in the presence of 12 borers per plant. This number of plants would have yielded 70.0 bushels in the absence of borers. Since the size of the ears was reduced 30.5 percent by 12 borers per plant, the yield of 7,798 plants would have been reduced 21.4 bushels by the smaller ears on all plants bearing marketable ears. The total reduction in yield would have been 36.0 bushels, which is 42.6 percent of 84.6 bushels, the yield in the absence of borers, or 3.55 percent per borer per plant. This loss is about as much as was suffered by Clarage and the open-pollinated group, even though the hybrids would have had more plants bearing marketable ears in the absence of borers.

EFFECT OF BORER POPULATION ON BREAKAGE OF THE CORN PLANT

The effect of different levels of corn borer population on the breakage of corn plants through feeding and tunneling within the stalk was a study incidental to the determination of the reduction in the amount and quality of the yield. A thorough study of the comparative ability of different strains to stand up under given borer populations requires the taking of data at intervals during the harvest season, especially after heavy winds. This was not possible in the present study, but it is believed that data from the Clarage variety taken from five localities during 4 years are fairly representative of an open-pollinated variety at harvest. It is believed also that sufficient data have been accumulated on open-pollinated varieties as a group and on hybrids to justify a comparison of the two groups.

COLLECTION AND CALCULATION OF DATA

Just before the harvest, during the last week of September and the first 2 weeks of October, the number of plants broken above the ear and the number broken below the ear were recorded for each plot. A plant broken both above and below the ear was recorded as broken below the ear. All breaks other than broken tassels were recorded, whatever the cause. The total number of plants per plot and the number of borers per plant at each infestation level were taken from the data used in the study of the quality and yield of the corn ears.

For the Clarage variety the number of plants broken below the ear was combined with the number broken above the ear to obtain the total number of broken plants, and the percentages of the plants broken were then calculated (table 3). Data from four levels of infestation from two plantings and five levels from nine plantings are given in table 3. Mean values are given for the plantings with five levels of infestation. For the groups of hybrids and of open-pollinated varieties complete data were obtained only on the number broken below the ear (table 4).

BREAKAGE OF CLARAGE AT VARIOUS INFESTATION LEVELS

For the Clarage variety it may be noted in table 3 that, as the number of borers per plant increased, both the total percentage of broken plants and the percentage of plants broken below the ear likewise increased. When the mean percentage of each variable was plotted against the average number of borers per plant for the five levels of infestation, a smooth curve could be passed through the points, as shown in figure 2. This curvilinear relation would necessarily follow from the way the data were taken. The number of broken plants, not the number of breaks per plant, was recorded. The ratio of the mean percentage of plants broken below the ear to the mean total percentage of broken plants, given in table 3, was about constant for the five levels of borer population, and averaged 59.0 percent

COMPARISON OF BREAKAGE IN HYBRIDS AND OPEN-POLLINATED VARIETIES

The average breakage of hybrids is compared with that of open-pollinated varieties in table 4, and the curved lines in figure 3 are fitted to these data. It may be noted, by extending the curves to the left, that about 3.5 percent of the hybrid plants and 10.5 percent of the open-pollinated plants would have been broken in the absence of borers. The curves also show that about 14.5 and 20.0 percent of the plants, respectively, were broken at a level of seven borers. Seven borers per plant would therefore have caused the breakage of 11.0 percent of the hybrid plants and 9.5 percent of the open-pollinated plants. Since the difference of 1.5 percent is of no practical importance, seven borers would have caused the breakage of an average of 10.3 percent of the plants, or 1.47 percent per borer per plant. In the absence of borers about 7.0 percent more of the open-pollinated than

TABLE 3.—*Breakage of plants of Clarage corn at different levels of population of the European corn borer, northwestern Ohio, 1929–32*

Date of planting	Locality	First level			Second level			Third level			Fourth level			Fifth level		
		Borers per plant	Total plants broken	Plants broken below ear	Borers per plant	Total plants broken	Plants broken below ear	Borers per plant	Total plants broken	Plants broken below ear	Borers per plant	Total plants broken	Plants broken below ear	Borers per plant	Total plants broken	Plants broken below ear
		Number	*Percent*	*Percent*	*Number*	*Percent*	*Percent*	*Number*	*Percent*	*Percent*	*Number*	*Percent*	*Percent*	*Number*	*Percent*	*Percent*
1929																
May 13	Sandusky	1.2	15.9	9.4	2.6	24.4	16.7	3.2	28.0	18.5	3.5	29.1	17.7	5.3	32.7	20.9
May 15	Huron	1.6	17.7	7.7	4.2	32.7	13.9	5.2	33.7	14.5	6.2	36.6	14.1	8.9	42.7	14.7
1930																
May 7	Sandusky	1.3	17.2	9.3	2.7	30.6	15.7	4.4	37.9	16.6	6.1	42.3	18.5			
May 9	Huron	1.1	13.1	5.0	2.6	20.6	9.3	4.7	33.2	13.0	5.8	36.4	14.1			
1931																
May 6	Sandusky	2.6	16.9	12.0	3.6	24.5	19.3	9.5	45.5	34.9	13.5	56.8	44.8	19.3	60.8	50.0
May 7	Huron	2.3	11.9	4.5	2.5	16.9	8.6	7.6	26.6	14.1	9.8	29.9	18.6	13.7	33.6	19.5
May 13	Toledo	.4	6.0	3.2	.9	13.3	8.9	5.2	30.9	14.2	7.8	38.8	20.9	9.9	43.6	24.5
1932																
May 11	Toledo	2.0	26.0	16.4	5.1	34.2	17.3	7.0	43.1	17.5	8.7	45.4	19.9	9.7	47.7	21.5
	Maumee	1.3	19.3	14.8	5.3	24.8	19.5	6.9	28.1	21.3	8.8	26.4	22.2	10.1	36.4	28.5
May 17	Sandusky	2.7	20.0	7.4	4.7	25.9	10.1	6.4	32.0	11.3	7.8	35.1	14.8	8.7	37.8	14.2
	Holgate	1.4	20.2	13.6	3.6	32.0	26.4	5.6	29.9	23.7	7.2	35.6	23.3	8.0	37.4	29.6
Mean (1929, 1931, and 1932)		1.7	17.1	9.9	3.6	25.4	15.6	6.3	33.1	18.9	8.1	37.1	21.8	10.4	41.4	24.8
Ratio of mean percentage of plants broken below ear to mean percentage of broken plants			57.9			61.4			57.1			58.8			59.9	

TABLE 4.—*Plants of hybrids and of open-pollinated varieties of corn broken below the ear at different levels of population of the European corn borer, Ohio, 1930–33*

Date of planting	Locality	Open-pollinated varieties						Hybrids					
		First level		Second level		Third level		First level		Second level		Third level	
		Borers per plant	Plants broken below ear	Borers per plant	Plants broken below ear	Borers per plant	Plants broken below ear	Borers per plant	Plants broken below ear	Borers per plant	Plants broken below ear	Borers per plant	Plants broken below ear
		Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
May 8, 1930	Sandusky	1.0	14.7	5.1	16.2	6.3	25.2	0.7	12.3	4.3	23.1	5.5	25.9
May 5, 12, 19, 25, 1931	do	2.9	12.3	8.9	19.5	13.9	18.0	2.4	6.9	8.8	12.7	14.2	15.2
May 20 and June 2, 1933	Maumee	.5	11.3	3.4	19.1	4.1	24.2	.4	2.4	2.5	6.3	3.5	11.0
	Holgate	.3	12.9	3.8	19.3	6.7	15.5	.2	1.9	2.9	4.9	4.1	5.3
Mean		1.2	12.8	5.3	18.5	7.8	20.7	.9	5.9	4.6	11.8	6.8	14.4

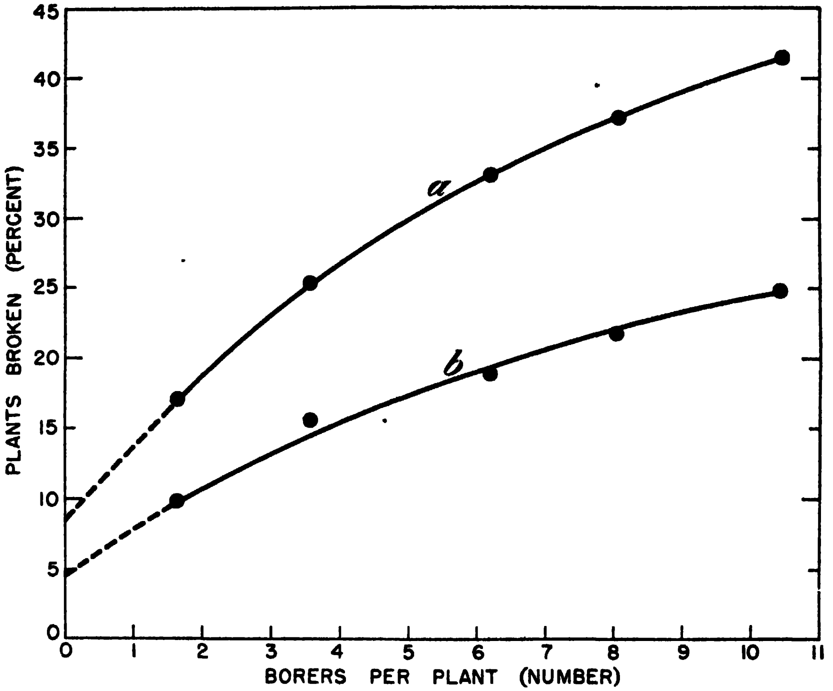

Figure 2.—Total percentage of broken plants (*a*) and percentage of plants broken below the ear (*b*) at different levels of borer infestation in the Clarage variety of corn.

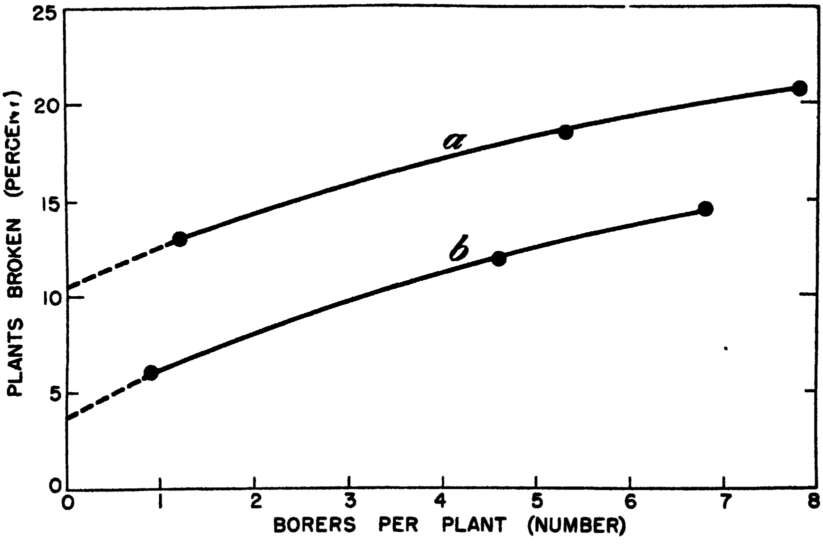

Figure 3.—Percentage of plants broken below the ear of open-pollinated (*a*) and hybrid (*b*) strains of corn at different levels of borer infestation.

of the hybrid plants would have been broken, and at a level of seven borers about 5.5 percent more, indicating that the hybrids were the more tolerant with respect to breakage. This was true, however, only because they stood up better without breakage in the absence of borers.

SUMMARY

The amount of injury to the quality and yield of the ears of field corn and the breakage of the stalks by infestations of the European corn borer (*Pyrausta nubilalis* (Hbn.)) were determined for hybrids and for the Clarage variety and other open-pollinated varieties in Ohio from 1929 to 1933. The various strains were subjected to different known levels of borer population induced by placing egg masses on the plants by hand. The regression of the factors studied on the borer population per plant was determined.

The reduction in the marketable yield occurred through an increase in the number of earless plants and plants bearing unmarketable ears and through a reduction in the size of the ear on all the plants bearing marketable ears.

The total reduction in marketable yield per acre per borer per plant was equivalent to the yield of about 292 hybrid or open-pollinated plants bearing marketable ears in the absence of borers. The size of the marketable ears was reduced about 2.50 percent per borer per plant in both the hybrids and the open-pollinated varieties.

On the basis of the marketable yield in the absence of borers, the marketable yield of the hybrids was reduced 2.99 percent and that of the open-pollinated varieties 3.64 percent per borer per plant. The hybrids were therefore more tolerant to the borer than the open-pollinated varieties. This advantage was due to the difference in the rate of increase per borer per plant in the number of earless plants and of plants bearing unmarketable ears per acre.

About 3.5 percent of the hybrid plants and 10.5 percent of the open-pollinated plants would have been broken below the ear in the absence of borers. Up to seven borers per plant the percentage of plants broken below the ear of both types of plants increased at the rate of about 1.47 per borer per plant. The hybrid plants therefore showed less breakage in the presence of borers only because they stood up better without breakage in the absence of borers.

In the Clarage variety 59.0 percent of all broken plants, excluding broken tassels, were broken below the ear on plants infested at levels up to 10 borers per plant.

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INHERITANCE OF RUST REACTION IN A CROSS BETWEEN THE FLAX VARIETIES BUDA AND J. W. S.[1]

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INTRODUCTION

The discovery of physiologic specialization in *Melampsora lini* (Pers.) Lév. in North and South America and in Europe (*3*, *4*, *12*, *13*)[2] has complicated the study of factors conditioning rust reaction in cultivated flax (*Linum usitatissimum* L.) as well as the development of rust-resistant varieties. On the basis of the reaction of 13 varieties of flax, 32 physiologic races have been differentiated, 24 from North and South America (*4*) and 8 from Europe (*12*). No variety is known to be immune from all the physiologic races, but 2 or more of the differential varieties are immune from any one race (*4*).

Henry (*9*) reported that an Argentine selection and the varieties Bombay and Ottawa 770B were immune from several collections of flax rust from North America and one from Europe. In a study of the inheritance of immunity from a North American collection of rust, in crosses involving these varieties, he found that immunity was conditioned by a single dominant factor in the varieties Bombay and Ottawa 770B and by either of two dominant factors in the Argentine selections. Myers (*10*) studied the inheritance of reaction to a field collection of flax rust and to a single physiologic race in 37 crosses involving 17 varieties or strains of flax. He found that reaction to both the collection and to the single physiologic race, except for minor differences probably attributable to environment, was similar and that he could account for the inheritance of immunity, near immunity, and resistance in the varieties he studied by assuming factors in two allelic series, L and M. L and M were dominant duplicate factors conditioning immunity; l^n and m^n were hypostatic to L and M and conditioned near immunity; and l^r and m^r were hypostatic to L and M and l^n and m^n and conditioned resistance. He was unable to interpret on a factorial basis segregations of crosses involving the varieties he designated as semiresistant, except by disregarding the difference between semiresistance and susceptibility in the progeny. With the exception of Bombay, which is susceptible to race 24, all the varieties studied by both Henry and Myers were susceptible to race 22.

No studies have been made in flax in which a variety resistant to one physiologic race of the rust fungus and susceptible to another race has been crossed with another variety reacting in a reverse manner to the two races. Such studies made with wheat varieties, involving resistance to *Puccinia graminis tritici* Eriks. and Henn., have shown that it is possible to obtain hybrids homozygous for resistance to both races, others homozygous for susceptibility to both races, and still others that react like the parent varieties (*6*, *7*, *8*, *11*). Aamodt (*1*), however, found that the factors for susceptibility, to physiologic race 1 of *P. graminis tritici* in Marquis, for moderate resistance in Kota, and for immunity in Kanred were allelic. Goulden, Neatby, and Welsh (*5*) concluded that the Marquis factors for high resistance to race 14 of *P. graminis tritici* were allelomorphic to the H-44 24 factors for moderate resistance to race 21, so that it was impossible to obtain a hybrid combining the resistance of both parents to these two races.

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[2] Italic numbers in parentheses refer to Literature Cited, p 387

The original object of the present study was to determine the inheritance of factors conditioning rust reaction in a cross between two varieties of flax used to differentiate physiologic races of the flax rust fungus and known to react reversely with respect to immunity and susceptibility to physiologic races 4 and 7. The investigation was expanded to include a study of the inheritance of additional types of rust reaction involving physiologic races to which (1) both parents were immune; (2) one parent was immune and the other (a) resistant, (b) semiresistant, (c) moderately susceptible, or (d) susceptible; (3) one parent was susceptible and the other moderately susceptible; and (4) both parents were susceptible. The work was done at the North Dakota Agricultural Experiment Station, Fargo, N. Dak.

MATERIAL AND METHODS

Among the crosses made in the greenhouse in 1934, involving the varieties then being used to differentiate the physiologic races of *Melampsora lini*, was one between Buda (C. I.[3] 270-1) and J. W. S. (C. I. 708-1). Buda is susceptible to race 4 and immune from race 7, and J. W. S. is immune from race 4 and susceptible to race 7.

TABLE 1.—*Reaction of Buda and J. W. S. to 24 physiologic races of the flax rust fungus*

Variety	Reaction[1] to physiologic race								
	20	1, 5, 6, 10 and 11	3, 14, 17, 18 and 23	2, 12, and 24	4, 8, 19, and 22	7	15	9 and 13	16 and 21
Buda	I	R+ to R−	SR	S	S	I	SR	S−	S
J. W. S.	I	I	I	I	I	S	S	S	S

[1] I=immune, R+=highly resistant, R=resistant, R−=moderately resistant, SR=semiresistant, S−=moderately susceptible, and S=susceptible.

The reaction of the two parental varieties is shown in table 1. The variety J. W. S. is either immune from or susceptible to each of the 24 physiologic races of the flax rust fungus that have been identified from North and South America. No difficulty has been experienced in determining its reaction even under adverse conditions for growth of flax. On the other hand, the reaction of Buda to different physiologic races ranges from immunity, through the intermediate stages, to susceptibility. Not only is the range of reaction of Buda extremely wide, but the intermediate reaction types are sensitive to environmental conditions, so that it is necessary to exercise caution in interpreting results. Environmental conditions that affect adversely the growth and vigor of the flax plant also depress rust development.

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[3] C. I. refers to accession number of the Division of Cereal Crops and Diseases.

All phases of the tests were conducted in the greenhouse during the late fall, winter, and early spring of the years 1934 to 1939 inclusive. Insects were kept under control at all times, and no evidence of natural crossing between the flax varieties in the greenhouse was observed. Early in these investigations it became evident that consistent rust readings could be obtained only on vigorously growing plants of about the same age, grown under similar environmental conditions. Topsoil (Fargo clay) from the experiment station fields was found superior to numerous variations of topsoil, sand, and compost, and it was used exclusively in these trials. The same soil was used repeatedly throughout the year but was steamed for 2 hours at 15 pounds' pressure prior to each sowing. As the study progressed, it was found that flax seedlings growing under the low light intensities and short days that prevail during the winter months were more thrifty if the greenhouse temperature was relatively low and the soil rather dry. A night temperature of 55° to 60° F. and a day temperature of 60° to 70° were found to be most satisfactory. A 16-hour light day was maintained by supplemental illumination from 200-watt Mazda lamps during the period of pustule formation.

Preliminary tests had demonstrated that as many as five flax plants could be grown to maturity in a single 6-inch pot and that these plants would produce sufficient seed for a statistical analysis of the progenies. Three F_1 seeds were sown per pot and five F_2 seeds. Two sets of F_2 plants were included in these tests. Each F_2 plant grown during the winter of 1935–36 was inoculated with physiologic races 4 and 7. In addition, some of the F_2 plants in this series were subsequently inoculated with race 3, 13, 19, or 20. Those grown during the winter of 1936–37 were inoculated with physiologic races 3, 4, and 7. The two F_1 plants that supplied the F_2 seed used in 1936–37 were different from the plants that supplied the seed used the previous year. Reciprocal crosses were studied, but since the differences in the reaction of the progenies were not statistically significant, the data have been combined.

Except for minor variations, the methods of inoculation previously developed (*3*) for the physiologic-race investigations were used throughout these tests. The urediospore inoculum of each race was increased on a susceptible variety, and the spores were collected in glass vials and stored at 4° C. When the seedlings were 2 to 3 inches tall and had 6 to 10 leaves they were inoculated by dusting urediospores, with a camel's-hair brush, on the unfolding leaves of the terminal bud. The plants were placed in a moist chamber, sprayed, and incubated overnight (18 hours) and returned to the greenhouse bench. Pustules broke the epidermis of susceptible varieties about 10 days after inoculation. In these trials it was possible to test each plant with from two to five physiologic races by reinoculating the plants with different races at intervals of 7 to 8 days and picking off the leaves previously inoculated as soon as the rust had developed sufficiently to differentiate the type of pustule. The type of infection produced on each plant was recorded and the plant tagged with colored yarn indicating a definite reaction to a specific race of rust.

As a result of severe rust infection, susceptible seedlings, if not killed directly, are weakened and often die before they mature seed. Since it was desired to obtain seed from each F_2 plant, care was taken to apply the inoculum to only a few leaves. These were less heavily inoculated than was customary in making physiologic-race determinations or in the subsequent tests of F_3 lines. Despite these precautions, some of the F_2 plants were so severely injured by the first test with a race to which they were susceptible that subsequent tests, with races to which they were resistant, were inaccurate. This difficulty was circumvented in the tests of the F_3 lines by using the races to which both parents were resistant in the earlier inoculations. The plants thus maintained a more normal growth, and complications due to reinfection were largely avoided.

Tests of F_3 lines were conducted in the same manner as were those of the F_2 lines except that eight plants were grown in a 4-inch pot, because it was usually not necessary to grow the plants to maturity. Four pots sown with eight seeds per pot formed the unit in testing F_3 lines. When doubt existed as to the accuracy or sufficiency of the test, additional units were sown if seed was available. In some cases checks on the accuracy of the F_3 rust readings were made by growing the F_3 plant to maturity and determining the rust reaction of the F_4 progeny.

For classifying rust reaction of flax plants, Myers (*10*) devised a key comprised of 11 classes, from 0 to 10 inclusive, based upon extent and type of infection. However, he was unable to follow it consistently in interpreting his results. For instance, seed from the Bison parent produced plants that ranged in reaction from class 2 to class 10 but gave no evidence of segregation. He stated that his class 4 and 5 reaction (semiresistant) was not very sharply differentiated genetically from the susceptible. To give a factorial interpretation of his results, he found it necessary to group classes 5 to 10 or 6 to 10 together as susceptible and classes 1, 2, and 3 as resistant. The inclusion of extent of infection in his classification appears to have accentuated difficulties in classifying the reaction of the individual plants grown under greenhouse conditions.

The following key to classes of host reaction and infection types as used in differentiating physiologic races of *Melampsora lini* (*3*) served as a guide for classifying the rust reaction of the individual plants in the present tests.

Classes of host reaction.	*Types of rust infection*
Nearly immune	(0) No uredia developed; hypersensitive flecks or necrotic lesions usually present, but sometimes there is no evidence of infection.
Resistant	(1) Uredia minute to small, rarely extending through the leaf, usually distinct and scattered in chlorotic to necrotic areas, but in some cases pustule formation is not accompanied by either chlorosis or necrosis of the surrounding leaf tissue
	(2) Uredia small to medium, associated with distinct necrosis of the leaf; may be scattered or may form crustlike aggregations in necrotic areas; if isolated, usually are surrounded by a necrotic zone.

Classes of host reaction—Continued.		*Types of rust infection*
Semiresistant	(3—)	Uredia variable; heavily inoculated areas necrotic, with arrested pustule development; medium to large pustules produced in healthy tissue adjacent to necrotic areas; pustules on stem and cotyledons small but with no evidence of hypersensitiveness.
Moderately susceptible	(3)	Uredia medium to large; well-developed but not compound; usually extending through the leaf to both surfaces; development somewhat retarded on older leaves and in heavily infected portions of the younger leaves; tissues adjacent to uredia may become more or less chlorotic as the pustules mature.
Susceptible	(4)	Uredia large and, if isolated, usually compound, extending through leaf to both surfaces; at first leaves show little chlorosis but later may become chlorotic and die prematurely.

In this key, the reaction class is determined by the type of pustule. In the subdivisions of the classes of host reaction, such as highly resistant, moderately resistant, moderately susceptible, and susceptible, consideration is given to the extent of infection as manifested by the relative number, size, and sporulation of pustules.

EXPERIMENTAL RESULTS

REACTION OF F_1 PLANTS AND PROGENY OF PARENT PLANTS

The F_1 plants of Buda × J. W. S. were immune from races 4 and 7 of *Melampsora lini*, indicating that the factors conditioning immunity were dominant in these varieties, as well as those studied by Henry (*9*) and Myers (*10*). Seed from the parent plants used in making the crosses also was tested. That from the Buda parent was homozygous for susceptibility to race 4 and for immunity from race 7, while that from the J. W. S. parent was homozygous for immunity from race 4 and for susceptibility to race 7.

REACTION OF F_2 PLANTS TO RACES 4 AND 7

The F_2 plants grown in the greenhouse during the winter of 1935–36 are classified in table 2 according to their reaction to races 4 and 7 of *Melampsora lini*. Results obtained from tests with races 3, 13, 19, and 20 were not sufficiently informative to warrant tabulation.

TABLE 2.—*Reaction of F_2 plants of Buda × J. W. S. to physiologic races 4 and 7 of the flax rust fungus*

Reaction of F_2 plants to race 4[1]	F_2 plants showing indicated reaction to race 7[2][3]			
	Immune	Resistant	Susceptible	Total
	Number	*Number*	*Number*	*Number*
Immune	209	18	33	260
Susceptible	88	0	0	88
Total	297	18	33	348

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[1] χ^2 for 3 immune : 1 susceptible to race 4=0.0153, P=0.90 to 0.95.

[2] χ^2 for 3 immune : 1 not immune from race 7=19.86, $P<0.01$.

[3] χ^2 for 15 resistant and immune : 1 susceptible to race 7=6.207, $P<0.01$.

Each of 165 F_2 plants tested was immune from race 20. All plants tested reacted the same to race 19 as to race 4. Tests with races 3 and 13 were not satisfactory, as the plants had become too old to consistently differentiate intermediate infection types.

A single dominant factor conditions immunity from race 4 in this cross as the segregation of 260 immune to 88 susceptible F_2 plants approximates a 3 to 1 ratio, P lying between 0.90 and 0.95. Apparently, a modifying factor was involved in the reaction to race 7, as some plants were distinctly resistant to this race, while others were either immune or susceptible. Even if all plants not immune from race 7 were considered as a unit, for a ratio of 3 immune to 1 not immune P was less than 0.01. This suggested that the test of the F_2 plants for reaction to race 7 was not accurate, or that reaction to this race was conditioned by more than one factor.

TESTS OF F_3 LINES WITH RACES 4 AND 7

The 348 F_3 lines of Buda × J. W. S. are classified in table 3 according to the reaction of the progenies to races 4 and 7 of *Melampsora lini*. The 88 F_2 plants that were susceptible to race 4 and immune from race 7 yielded F_3 progenies homozygous for the same reaction. This confirmed the accuracy of the test of the F_2 plants for their reaction to race 4. Of the 260 lines descended from F_2 plants immune from race 4, 175 segregated for susceptible and immune plants and 85 were homozygous for immunity to that race. The fit of the observed ratio to the theoretical 1:2:1 for reaction of the 348 F_3 lines to race 4 was close, P lying between 0.95 and 0.98.

TABLE 3. *Reaction*[1] *of F_3 lines of Buda × J. W. S. to physiologic races 4 and 7 of the flax rust fungus*

Parental F_2 plants			F_3 progenies having indicated reaction to races 4 and 7						
Number	Reaction to race		All I (race 4)			I and S (race 4)			All S (race 4)
	4	7	All R (race 7)	R, SR, S (race 7)	All S (race 7)	I and R (race 7)	I, R, SR, S (race 7)	I and S (race 7)	All I (race 7)
			Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
209	I	I	21	13		57	77	41	
18	I	R		18					
33	I	S		6	27				
88	S	I							88
Total observed [2]			21	37	27	57	77	41	88
Expected, 1:2:1:2:4:2:4			21.75	43.5	21.75	43.5	87	43.5	87
Observed [3]			21	37	27	134		41	88
Expected, 1:2:1:6:2:4			21.75	43.5	21.75	130.5		43.5	87
Observed [4]				85			175		88
Expected, 1:2:1				87			174		87

[1] See footnote 1, table 1.
[2] $\chi^2=7.758$; $P=0.20$ to 0.30.
[3] $\chi^2=2.513$; $P=0.70$ to 0.80.
[4] $\chi^2=0.063$; $P=0.95$ to 0.98.

The progeny of each of the 51 F_2 plants that had not been immune from race 7 was homozygous for immunity from race 4 and for some degree of susceptibility to race 7. In addition, the progenies of 34 of the 209 F_2 plants that had been classed as immune from both races 4 and 7 were immune from race 4 and were either homozygous for resistance or segregated for resistance, semiresistance, and susceptibility to race 7. These 34 F_2 plants had resisted infection probably because of the use of insufficient inoculum and because of the adverse effect on the vigor of the host of low light intensity during a prolonged cold period when the glass of the greenhouse was continuously coated with frost. The progenies of 6 of the 33 F_2 plants that had been classed as susceptible to race 7 segregated for susceptible and resistant to semiresistant plants. Apparently the factor conditioning resistance to race 7 in this cross was not completely dominant and was sensitive to environment, as the reaction of heterozygous plants sometimes approached the moderately susceptible. Of the 85 F_3 lines that were homozygous for immunity from race 4, 27 were homozygous for susceptibility, 37 segregated for susceptible, semiresistant, and resistant plants, and 21 were pure for resistance to race 7.

The 175 F_3 lines heterozygous for reaction to race 4 were heterozygous also for reaction to race 7. All plants of 41 lines were either susceptible to one race and immune from the other or immune from both. Each of the remaining 134 lines contained plants having a distinctly resistant reaction to race 7. In 77 of these lines one or more plants were highly susceptible to race 7, and in the remaining 57 lines all plants were either resistant or immune. Since each of the 10,540 F_3 plants as well as each of the 348 F_2 plants tested was immune from either race 4 or race 7 or from both, the factors conditioning immunity from race 4 in J.W.S. and from race 7 in Buda apparently were in one allelic series.

These results suggest that Buda also carries a factor for resistance to race 7, independent of and hypostatic to the immune factor. If this hypothesis is correct, the ratio of F_3 lines for reaction to race 7 would approximate 1 resistant to 2 resistant and susceptible to 1 susceptible to 2 immune and resistant to 4 immune, resistant, and susceptible to 2 immune and susceptible to 4 immune.

The fit of the observed ratio to the theoretical was good (table 3), P lying between 0.20 and 0.30. The greatest deviation from the theoretical distribution was in groups heterozygous to race 4 and containing plants having a resistant reaction to race 7. There were too many lines containing only resistant and immune plants and too few having susceptible, resistant, and immune plants. The criterion for separation of these 2 groups was the occurrence of plants susceptible to race 7. In a sample of 32 plants the theoretical distribution for reaction to race 7 in one group was 8 resistant to 24 immune and in the other, 2 susceptible to 6 resistant to 24 immune. The theoretical expectation of obtaining no susceptible plant in a random sample of 32 from the latter group is 0.134. Therefore, it is probable that approximately 11 of the 57 F_3 lines that had only resistant and immune plants when tested with race 7 would have had susceptible individuals had a larger sample been used. This is indicated by the close approach to the theoretical ratio of F_3 lines when these 2 groups are combined. P for a 1:2:1:6:2:4 distribution lies between 0.70 and 0.80.

REACTION OF F_3 PLANTS TO RACES 3, 4, AND 7

Tests of some of the F_3 lines susceptible to race 4 indicated a segregation for resistance and susceptibility to race 3. As adequate seed for additional tests of a number of the F_3 lines was not available, a second series of infection studies was made in which races 3, 4, and 7 were used on 237 F_2 plants. The results are shown in table 4.

As in the first series of infection studies, the fit of the F_2 plants to a ratio of 3 immune to 1 susceptible to race 4 was good, *P* lying between 0.30 and 0.50. The inoculation with race 4 preceded that with race 3, and as a consequence all plants in any degree susceptible to the latter race had been weakened by the attack of race 4 and the subsequent stripping of infected leaves. This fact, together with the increased age of the plants at the time of inoculation and the adverse climatic conditions that prevailed during the period that race 3 developed in the plants, obscured differences in infection types. The segregation for intermediate reaction to race 3 was indefinite, but all plants that had been susceptible to race 4 were attacked in varying degrees of severity by race 3, and all that had been immune from race 4 were also immune from race 3.

The test of the F_2 plants with race 7, although not wholly satisfactory, was better than the earlier one. The fit for a ratio of 15 immune and resistant to 1 susceptible was satisfactory, *P* lying between 0.30 and 0.50. However, *P* for a ratio of 12 immune to 3 resistant to 1 susceptible was less than 0.01. Unless the infection is severe enough to stunt or kill susceptible plants, some that are intermediate in reaction to race 7 apparently escape or resist infection.

TABLE 4.—*Correlation table showing reaction of F_2 plants of Buda × J. W. S. to physiologic races 3, 4, and 7 of the flax rust fungus*

F_2 plants having indicated reaction to—					
Race 3[1]	Race 4[2]	Race 7[3]			
		I	SR to R	S	Total
		Number	*Number*	*Number*	*Number*
I	I	140	15	18	173
R— to S—	S	28			28
S	S	36			36
Total		204	15	18	237

[1] See footnote 1, table 1.

[2] χ^2 for 3 I : 1 S to race 4=0.508, P=0.30 to 0.50.

[3] χ^2 for 15 I and SR to R : 1 S to race 7=0.612, P=0.30 to 0.50.

TESTS OF F_3 LINES WITH RACES 3, 4, AND 7

The rust reaction of 26 to 157 plants from each of the 236 F_3 lines was determined by successive inoculation with races 3, 4, and 7 as previously outlined. Adverse growing conditions, especially during January and February, interfered with the accurate reading of intermediate reaction to races 3 and 7. Later, during the spring months, when conditions in the greenhouse were more favorable for growth of the host and for rust development, tests of lines having plants of doubtful reactions were repeated, and satisfactory results were obtained. When grouped according to the diversity of reaction of each F_3 progeny to races 3, 4, and 7, the 236 F_3 lines fall into the 9 classes listed in table 5.

TABLE 5.- *Reaction*[1] *of F_3 lines of Buda × J. W. S. to physiologic races 3, 4, and 7 of the flax rust fungus*

Reaction of F_3 plants to: Race 3	Race 4	Race 7	F_3 lines	F_3 plants	Reaction of F_3 plants to: Race 3	Race 4	Race 7
			Number	*Number*			
S, SR, R	S	I	22	910	SR	S	I
S, SR, R	S	I	27	258	S	S	I
				856	SR	S	I
S, SR	S	I	15	616	S	S	I
Total			64	2,640			
I	I	I	25	298	SR	S	I
				637	I	I	I
				273	I	I	R
I	I	I	66	193	S	S	I
				630	SR	S	I
				1,904	I	I	I
				583	I	I	R
				229	I	I	S
I	I	I	32	622	S	S	I
				1,286	I	I	I
				667	I	I	S
Total			123	7,322			
I	I	I, R–	11	373	I	I	R
I	I	SR, R, I	21	1,004	I	I	R
				320	I	I	S
I	I	S	17	982	I	I	S
Total			49	2,679			
Grand total			236	12,641			

[1] See footnote 1, table 1.

None of the 12,641 plants in this test was susceptible both to race 7 and to either of races 3 or 4. This fact, as well as the behavior of the 348 F_3 lines as presented in table 3, substantiates the conclusion that the factors conditioning immunity in this cross are dominant and allelic. Of the 236 F_3 lines tested, 64 were homozygous for susceptibility to race 4 and for immunity from race 7, 123 were heterozygous, having plants immune from both races, and 49 were homozygous for immunity from race 4 and for some degree of susceptibility to race 7. This observed ratio fits satisfactorily the theoretical 1 : 2 : 1, *P* lying between 0.30 and 0.50. The reaction of the F_3 population in the 3 groups of lines having characteristic reaction to race 4 indicates that the semiresistant reaction to race 3 and the resistant reaction to race 7 are incompletely dominant to susceptibility (fig. 1, *A*, *a*, *b*; *B*, *a*, *b*) and are conditioned by factors independent of and hypostatic to the factors conditioning immunity. Of the 64 lines susceptible to race 4 and immune from race 7, 15 were homozygous for susceptibility to race 3; 27 were heterozygous, having both semiresistant and susceptible plants; and 22 were homozygous for semiresistance. The *P* for fit of this distribution to the theoretical 1 : 2 : 1 lay between 0.20 and 0.30. Of the 123 lines heterozygous for immunity, 25 were homozygous for the resistant factors, as all plants not immune from races 3, 4, and 7 were either semiresistant to race 3 and immune from race 7 or resistant to race 7 and immune from race 3; 66 were heterozygous for the resistant factors; and 32 were homozygous for the recessive susceptibility factors, as all plants not immune from both races 3 and 7 were either susceptible to race 3 and immune from race 7 or susceptible to race 7 and immune from race 3. The fit of the observed ratio of F_3 lines to the expected 1 : 2 : 1 for segregation of the resistant factor in this heterozygous immune group was good, *P* lying between 0.30 and 0.50. Of the 49 F_3 lines immune from races 3 and 4, 11 were homozygous for resistance to race 7; 21 were heterozygous, having both susceptible and resistant plants; and 17 were homozygous for susceptibility. The fit of the observed ratio to the calculated 1 : 2 : 1 is satisfactory, *P* lying between 0.20 and 0.30.

Myers (*10*) concluded from his studies that a single dominant factor conditioned immunity from a collection of rust and from race 4 in Ottawa 770B and that C. I. 438 carried the same factor for immunity as Ottawa 770B. However, C. I. 438 is susceptible to and Ottawa 770B is immune from races 19 and 20; so it is probable that the factors conditioning immunity in these 2 varieties are allelic rather than identical. Both Ottawa 770B and C. I. 438 were immune from the strains of rust used by Myers, so that this difference in reaction of the varieties was not discernible in his tests. Preliminary studies of the writer have indicated that the factor conditioning immunity from 18 races, carried by J. W. S., is allelic to the factor of Ottawa 770B conditioning immunity from 23 races. Myers designated the pair of immunity factors of Ottawa 770B as *LL*. As indicated in table 6, it is proposed to designate the factors conditioning immunity from race 7 in Buda as $L^1 L^1$ and the factors conditioning immunity from race 4 in J. W. S. as $L^2 L^2$. The relation of the factors conditioning semiresistance to race 3, carried by Buda, to Myers' *MM* series of allels conditioning rust reaction is not known. Until this relation is de-

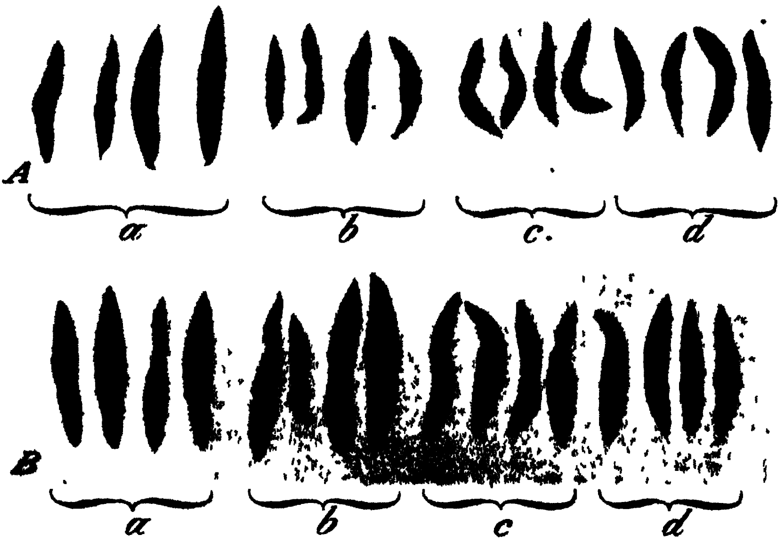


FIGURE 1.—Reaction of homozygous and heterozygous F_3 segregates from the hybrid Buda × J. W. S. to physiologic races 3 and 7, showing the dominance of the immune factors L^1 and L^2 and the incomplete dominance of the resistant factor *R*. *A*, Race 3: *a*, L^1L^1rr; *b*, L^1L^1Rr; *c*, L^1L^1RR; and *d*, L^1L^2rr. *B*, Race 7: *a*, L^2L^2rr; *b*, L^2L^2Rr; *c*, L^2L^2RR; and *d*, L^1L^2rr. Natural size.

termined, it is proposed to designate the semiresistant factors carried by Buda as *RR* and the recessive allels in J. W. S. as *rr*. On the basis of this hypothesis, the F_3 lines are grouped in table 6 according to the F_2 genotypes, as indicated by the reaction of the F_3 population to races 3, 4, and 7. Values of χ^2 for fit to the expected ratios for each of the segregating lines were calculated, and the value of *P* was determined for each. Of the 171 F_3 lines segregating for reaction to races 3 or 7, the *P* for 1 line lay between 0.01 and 0.02, the *P* for 5 lines lay between 0.02 and 0.05, and the *P* for 165 lines was greater than 0.05. The formula given by Fisher (*2*), $\sqrt{2\chi^2}-\sqrt{2n-1}$, when applied to the sum of the χ^2 values of the 171 segregating lines, gave a normal deviate of 1.478 + 1, showing that the data are in accord with the hypothesis.

TABLE 6. *Segregation for F_2 genotypes in Buda × J. W. S. as indicated by the reaction of F_3 lines to physiologic races of the flax rust fungus*

Variety or hybrid	F_2 genotype	Theoretical ratio	F_3 lines segregating		Ratio of F_3 plants	Reaction² of F_3 plants to		
			Expected	Observed		Race 3	Race 4	Race 7
			Number	*Number*				
Buda					All	SR	S	I
J. W. S.					All	I	I	S
F_2	L^1L^1RR	1	14.8	22	All	SR	S	I
	L^1L^1Rr	2	29.6	27	1	S	S	I
					3	SR	S	I
	L^1L^1rr	1	14.8	15	All	S	S	I
	$L^1L\,RR$	2	29.6	25	1	SR	S	I
					2	I	I	I
					1	I	I	R
	$L^1L\,Rr$	4	59.3	66	1	S	S	I
					3	SR	S	I
					8	I	I	I
					3	I	I	R
					1	I	I	S
	$L^1L\,rr$	2	29.6	32	1	S	S	I
					2	I	I	I
					1	I	I	S
	$LL\,RR$	1	14.8	11	All	I	I	R
	$LL\,Rr$	2	29.6	21	3	I	I	R
					1	I	I	S
	$LL\,rr$	1	14.8	17	All	I	I	S

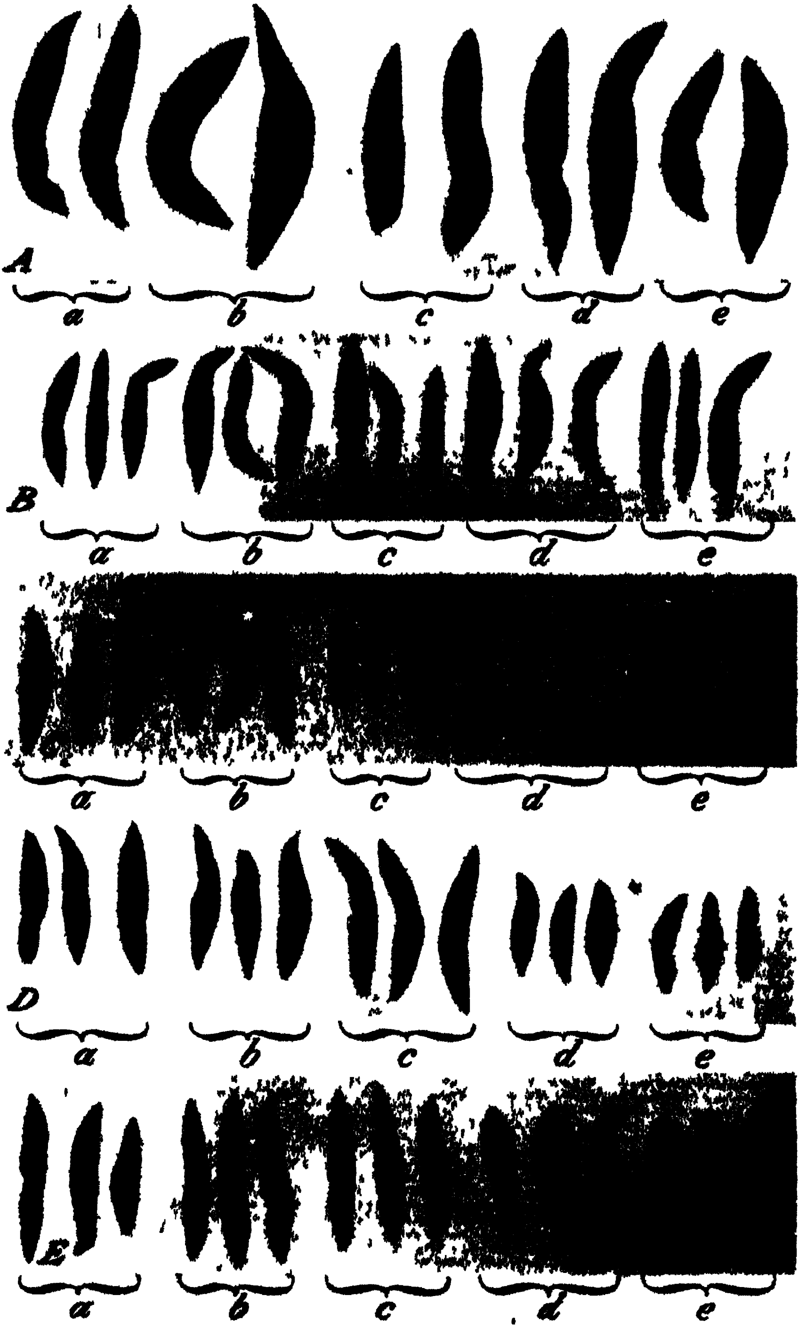
[1] $\chi^2 = 9.252$; $P = 0.30$ to 0.50.

[2] See footnote 1, table 1.

In table 6 is recorded the number of F_3 lines descended from each of the theoretical F_2 genotypes, as indicated by the reaction of the F_3 population to physiologic races 3, 4, and 7, and the expected distribution of F_3 plants from each F_2 genotype for reaction to these races. The fit of the F_3 lines to a 1:2:1:2:4:2:1:2:1 distribution was good. For the 236 lines, *P* lay between 0.30 and 0.50, indicating that the data are in agreement with the hypothesis.

REACTION OF F_3 GENOTYPES TO RACES DIFFERENTIATED BY BUDA AND J. W. S.

The correlation of reaction to various races of the flax rust fungus was studied by inoculating plants of F_3 lines, whose reaction had been successively determined to races 3, 4, and 7, with one or more of the following races: 1, 2, 5, 8, 9, 10, 16, 19, 20, 21, and 22. Although a wide range of reaction combinations was included in these tests, there was no indication of the presence of factors conditioning rust



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reaction other than those affecting races 3, 4, and 7. Since the age of the plant influences the expression of the intermediate infection types, these supplemental tests on 40- to 60-day-old plants were not strictly comparable with those of races 3, 4, and 7 on 20- to 30-day-old plants. To obviate this, a line was selected for study from each of the four genetically different groups that were homozygous for factors conditioning rust reaction, as indicated by the tests with races 3 and 7. Line 7, L^1L^1RR, was similar to the Buda parent and line 8, L^1L^1rr, possessed the factors conditioning immunity from race 7 but lacked the factors conditioning semiresistance to race 3. Line 575, L^2L^2rr, possessed the factors conditioning immunity from race 4 and susceptibility to race 7 of the J. W. S. parent, and line 584, L^2L^2RR, had in addition to the immune factors of the J. W. S. parent the factors that conditioned semiresistance to race 3 in Buda. The reaction of the parents and of these four F_3 lines to a race representative of each group of races differentiated by the reaction of Buda and J. W. S. is given in table 7.

TABLE 7.—*Seedling reaction to 9 physiologic races of the flax rust fungus of Buda, J. W. S., and F_3 lines of Buda × J. W. S. homozygous for factors conditioning rust reaction*

Variety or F_3 line	Genotype	Seedling reaction[1] to physiologic race—								
		20	5	1	3	2	4	7	9	16
Buda	L^1L^1RR	I	R+	R+ to R	SR	S–	S	I	S	S
J. W. S.	L^2L^2rr	I	I	I	I	I	I	S	S	S
F_3 line:										
7	L^1L^1RR	I	R+	R+ to R–	SR	S–	S	I	S–	S
8	L^1L^1rr	I	R to R–	R to S	S	S	S	I	S– to S	S
575	L^2L^2rr	I	I	I	I	I	I	S	S	S
584	L^2L^2RR	I	I	I	I	I	I	R	S– to S	S

[1] See footnote 1, table 1.

REACTION TO RACE 20

Both parent varieties, Buda and J. W. S., have been consistently immune from race 20. During these studies 165 F_2 plants as well as numerous F_3 lines, including lines heterozygous as well as those homozygous for factors conditioning rust reaction, were tested and were invariably immune. However, the immune reaction of plants having the J. W. S. factor for immunity (L^2L^2) was not identical with that of plants having the Buda factor (L^1L^1) and was affected by environmental conditions. Plants having the immune factors of the J. W. S. parent tested in February, when light is deficient, developed distinct necrotic flecks and a tendency toward chlorosis in heavily inoculated portions of the leaves (fig. 2, *A*, *a*, *c*, *e*). In similar tests, conducted during the latter part of March under more

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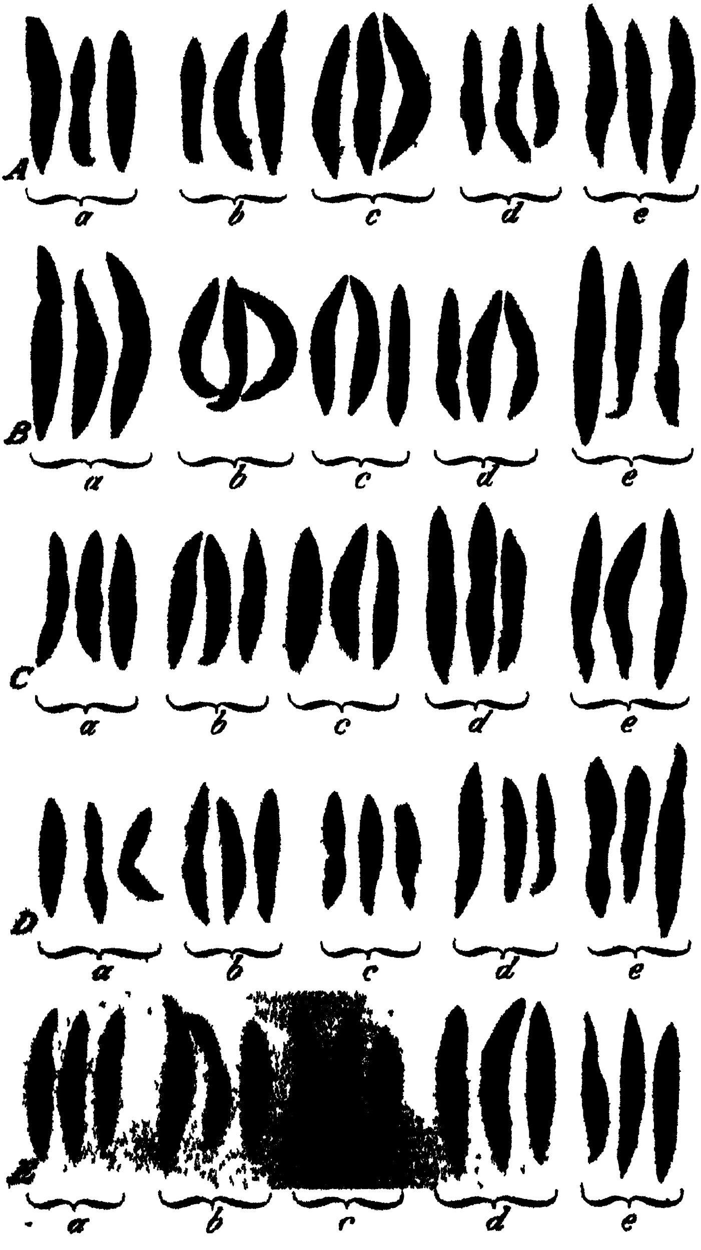
FIGURE 2. Reaction of the parent varieties Buda (L^1L^1RR) and J. W. S. (L^2L^2rr) and F_3 hybrid segregates of indicated genotype to physiologic races of *Melampsora lini* differentiated by Buda and J. W. S. *A*, Race 20, reaction in midwinter *a*, J. W. S.; *b*, Buda; *c*, L^2L^2RR; *d*, L^1L^1rr; and *e*, L^2L^2rr. *B*, Race 20, reaction in spring: *a*, L^1L^1rr; *b*, L^1L^1RR; *c*, L^2L^2RR; *d*, Buda; and *e*, J. W. S. *C*, Race 5: *a*, L^1L^1rr; *b*, L^1L^1Rr; *c*, L^1L^1RR; *d*, Buda; and *e*, J. W. S. *D*, Race 1: *a*, L^1L^1rr; *b*, L^1L^1Rr; *c*, L^1L^1RR; *d*, Buda; and *e*, J. W. S. *E*, Race 17. *a*, L^1L^1rr; *b*, L^1L^1Rr; *c*, L^1L^1RR; *d*, Buda; and *e*, J. W. S. *A*, × 1.5; *B* to *E*, natural size.



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favorable growing conditions, necrotic lesions were absent and the principal evidences of inoculation were the distortion and slight stunting of heavily inoculated leaves and the gray mycelial weft left by the germ tubes (fig. 2, *B*, *c*, *e*). In the February tests, the response to inoculation with race 20 of plants having the Buda factor for immunity was a slight flecking and distortion of the heavily inoculated portions of the leaves (fig. 2, *A*, *b*, *d*), but in tests during the latter part of March the leaves became distorted and developed chlorotic to necrotic lesions (fig. 2, *B*, *a*, *b*, *d*). Although no rudimentary uredia have been observed in these chlorotic to necrotic areas, this response was suggestive of the reaction of Buda to race 5 (fig. 2, *C*, *d*). If failure to form uredia is the criterion for immunity from rust, then immunity is dependent not on exclusion of the pathogen but on the limitation of its development. It is not improbable that, under favorable environmental conditions, the development of race 20 might be extended to permit the formation of rudimentary uredia in these chlorotic to necrotic areas.

REACTION TO RACES 1 AND 5

Race 1 is a composite of races of restricted pathogenicity as measured by ability to attack the rust-differentiating varieties (*3*). The reaction of Buda to the different strains of race 1 varies from high resistance through intermediate types to moderate resistance. The strains to which Buda is highly resistant (*R*+) appear to be distinct from those to which it is moderately resistant (*R*−), but their separation is complicated by the occurrence of intermediate strains, so that impracticably rigid control of environmental conditions is required to secure consistent differentiation.

Immunity from all strains of race 1 and from race 5 is conditioned by the L^2 factor of J. W. S. (fig. 2, *C*, *c*; *D*, *c*). Both factors that condition rust reaction in Buda are operative to races 1 and 5. Line 8, L^1L^1rr, was resistant to race 5 (fig. 2, *C*, *a*) and to strains of race 1 to which Buda was highly resistant, and was moderately susceptible (fig. 2, *D*, *a*) to the strains of race 1 to which Buda was moderately resistant. Line 7, L^1L^1RR, was more resistant to races 5 (fig. 2, *C*, *e*) and 1 (fig. 2, *D*, *e*) than was line 8; and heterozygous plants, L^1L^1Rr, were intermediate in reaction (fig. 2, *C*, *b*; *D*, *b*).

REACTION TO RACES 3 AND 17

The semiresistant reaction of Buda differentiates races 3 and 17 from races 5 and 1, respectively. Line 8, L^1L^1rr, was susceptible both to race 3 (fig. 3, *A*, *a*) and to race 17 (fig. 2, *E*, *a*). However, the pustules of the latter race, although more numerous, were smaller, and the infected leaves tended to die prematurely, indicating that

FIGURE 3. Reaction of the parent varieties Buda (L^1L^1RR) and J. W. S. (L^2L^2rr) and F_3 hybrid segregates of indicated genotype to physiologic races of *Melampsora lini* differentiated by Buda and J. W. S. *A*, Race 3: *a*, L^1L^1rr; *b*, L^1L^1RR; *c*, L^2L^2RR; *d*, Buda; and *e*, J. W. S. *B*, Race 7: *a*, L^1L^2rr; *b*, L^2L^2RR; *c*, L^1L^1rr; *d*, Buda; and *e*, J. W. S. *C*, Race 4: *a*, L^1L^1rr; *b*, L^1L^1RR; *c*, L^2L^2RR; *d*, Buda; and *e*, J. W. S. *D*, Race 9: *a*, L^1L^1rr; *b*, L^1L^1RR; *c*, L^2L^1RR; *d*, Buda; and *e*, J. W. S. *E*, Race 16: *a*, L^1L^1rr; *b*, L^1L^1RR; *c*, L^2L^2RR; *d*, Buda; and *e* J. W. S. Natural size.

there was some degree of incompatibility between host and pathogen and that the L^1L^1 factors, while ineffective to race 3, were not wholly so to race 17. The reaction of line 7, L^1L^1RR, and plants heterozygous for the resistant factor, L^1L^1Rr, to races 3 and 17 also varied. Isolated pustules of race 3 were large and the copiously inoculated regions of the leaves became rather sharply necrotic (fig. 3, *A*, *b*, *d*). The pustules of race 17 were small and numerous, and the well-inoculated portions of the leaves (fig. 2, *E*, *b*, *c*, *d*), although dying prematurely, were characterized by a chlorosis rather than the sharp necrosis of race 3.

REACTION TO RACE 2

The reaction of F_3 lines of various genotypes to race 2 was similar to their reaction to race 3, except that genotype L^1L^1RR (line 7) was semiresistant to race 3 and moderately susceptible to race 2.

REACTION TO RACES 4, 8, 19, AND 22

Extensive tests of F_2 plants and F_3 lines were made with races 4, 8, 19, and 22, to which Buda is susceptible and J. W. S. immune. Immunity from these races was dominant and was conditioned by the factor L^2 (fig. 3, *C*, *c*, *e*). The susceptibility of seedlings of Buda and of F_3 line 7, L^1L^1RR (fig. 3, *C*, *b*, *d*), shows that neither the L^1 nor the R factors condition resistance to these races. However, when infected by races 4 or 8, older plants of F_3 line 7, especially when grown under adverse conditions, had fewer and smaller pustules and showed a greater tendency toward chlorosis and premature death of infected leaves than did plants of F_3 line 8, L^1L^1rr (fig. 3, *C*, *a*). All plants not having the immune factor L^2 were highly susceptible to races 19 and 22, even when grown under adverse conditions.

REACTION TO RACE 7

Buda is immune from and J. W. S. susceptible to race 7 (fig. 3, *B*, *d*, *e*), the reverse of the reaction of these parent varieties to races 4, 8, 19, and 22. Immunity from race 7 was conditioned by the dominant factor L^1 (fig. 3, *B*, *c*, *d*). The factors RR, which condition semiresistance to race 3 in Buda, condition resistance to race 7 (fig. 3, *B*, *b*).

REACTION TO RACE 15

This race was lost and no tests were made with it.

REACTION TO RACE 9

Extensive tests of F_3 lines were made with race 9. All seedling plants regardless of genotype were susceptible, but to varying degrees, as shown in table 7. The J. W. S. parent (fig. 3, *D*, *e*) and line 575, L^2L^2rr, were susceptible. Line 584, L^2L^2RR (fig. 3, *D*, *c*), was distinctly less susceptible than line 575. Line 8, L^1L^1rr (fig. 3, *D*, *a*), appeared to be slightly less susceptible than line 584; and line 7, L^1L^1RR, and the Buda parent were classed as moderately susceptible (fig. 3, *D*, *b*, *d*). Plants of genotype L^2L^2RR and L^1L^1RR developed a type of mature-plant resistance in which relatively few pustules developed, even when the leaves were heavily inoculated.

REACTION TO RACES 16 AND 21

The F_3 plants containing the factors conditioning rust reaction in all possible combinations, heterozygous as well as homozygous, were invariably susceptible to races 16 and 21 (fig. 3, *E*). Plants having the factors L^2L^2rr, conditioning rust reaction of the J. W. S. parent (fig. 3, *E, e*), were somewhat more susceptible than heterozygous ones or those having the L^1 and R factors (fig. 3, *E, a, b, c, d*), as indicated by pustule vigor, ability to produce an abundance of spores for a prolonged period, and compatibility between host and parasite.

REACTION OF HETEROZYGOUS PLANTS

Under favorable conditions for growth of flax and for rust development, the effect of host heterozygosity on development of rust by races producing intermediate infection types was evident. Immunity from rust was dominant, and plants heterozygous for the immune factors L^1L^2 were immune from all races from which either parent was immune (fig. 1, *A, d; B, d*). The incomplete dominance of the resistance factor R was apparent in those lines homozygous for the factors conditioning immunity from either race 4 or race 7 and heterozygous for the factors conditioning semiresistance to race 3 (fig. 1, *A, b;* fig. 1, *B, b;* fig. 2, *D, b;* fig. 2, *E, b*). For example, F_3 line 431, L^1L^1Rr, in one test had 7 plants susceptible, 15 moderately susceptible, and 7 semiresistant to race 3 (fig. 1, *A, a, b, c*). Line 463, L^2L^2Rr, had 5 plants susceptible, 19 semiresistant, and 8 resistant to race 7 (fig. 1, *B, a, b, c*). These resistant plants of line 463 were grown to maturity, and, as the F_4 progeny was homozygous for resistant reaction to race 7, it was evident that differences in degree of resistance of the F_3 plants had had a genetic basis. However, it was only during periods exceptionally favorable for growth of the plant and for rust development that variations of the intermediate infection types could be considered as reliable indicators of genotypic differences.

DISCUSSION

The data presented serve to emphasize that in developing a program for breeding rust-immune varieties of flax it is important to know the inheritance of certain plant characters and to understand the interaction of the genes that condition rust reaction in the parent varieties as well as the relation between these factors and the physiologic races of the flax rust fungus.

Previous investigations had indicated that the inheritance of immunity from flax rust was conditioned by one or two dominant factors (*9, 10*). In the present investigations, however, it was found that, while immunity from specific races of rust was conditioned by dominant genes, the dominants carried by Buda and J. W. S. and controlling their immunity and susceptibility to two physiologic races are allelic. Consequently, no segregates could be obtained that were homozygous for both of the dominant allels conditioning immunity from the two races of rust. Likewise, no segregates were obtainable that were susceptible to both of the races.

Obviously, in a region where both races 4 and 7 occur, it would be futile to attempt to obtain a rust-immune variety of flax by crossing Buda and J. W. S., although each variety is immune from one of the races and neither variety is susceptible to both. On the other hand, the existence of the allelic series of immunity factors simplifies the problem of breeding for immunity from certain physiologic races of the flax rust fungus. Thus, Ottawa 770B has been immune from all North American and European rust collections (*4*, *12*) and J. W. S. has been immune in South America (*14*). In a cross between these varieties the factors *LL* of Ottawa 770B condition immunity from North American and European races and susceptibility to a South American race (race 22), while the allelic factors L^2L^2 of J. W. S. condition immunity from South American races and susceptibility to certain North American and European races. Since susceptibility to rust is recessive, F_2 plants susceptible to race 22 are homozygous for the factors *LL* conditioning immunity from North American and European races of rust, and F_2 plants susceptible to any one of races 7, 9, 13, 15, 16, or 21 are homozygous for the factors L^2L^2 conditioning immunity from South American races. In either case, three-fourths of the plants could be discarded in the F_2 generation and subsequent tests could be confined to material homozygous for the pair of factors conditioning immunity from the races of rust with which the investigator is concerned.

The use of a line having the genotype L^1L^1rr, in addition to the regular series of differential varieties, is helpful in the identification of races of *Melampsora lini* differentiated by the semiresistant reaction of Buda. To these races Buda may be somewhat resistant in tests conducted under unfavorable conditions. Plants of genotype L^1L^1rr are susceptible, even under adverse growing conditions, to races to which Buda is semiresistant.

The reaction of Buda to the different races of the flax rust fungus, varying by almost imperceptible stages from immunity (no evidence of the formation of uredia) to susceptibility, is suggestive of the involvement of multiple factors either in the host or in the pathogen. Since no evidence was obtained that more than two factors are concerned in conditioning rust reaction in Buda, these studies suggest that the variable reaction of this variety is due to the interaction of quantitative factors inherent in the rust fungus.

SUMMARY

The inheritance of factors conditioning reaction to the flax rust fungus was studied in a cross between Buda and J. W. S., two varieties possessing differential reactions to physiologic races of *Melampsora lini*. J. W. S. is immune from 18 of the 24 races that have been identified from North and South American collections of the flax rust fungus and susceptible to races 7, 9, 13, 15, 16, and 21. Buda is immune from races 7 and 20; resistant to races 1, 5, 6, 10, and 11; semiresistant to races 3, 14, 15, 17, 18, and 23; moderately susceptible to races 2, 9, 12, 13, and 24; and susceptible to races 4, 8, 16, 19, 21, and 22.

The F_1 generation was immune from all races from which either parent was immune, indicating that immunity was dominant. Susceptible genotypes could be identified in the F_2 generation, but those having intermediate infection types required further tests of F_3 behavior.

Each F_3 line that was attacked by race 7 was homozygous for immunity from races 3 and 4. Conversely, each F_3 line attacked by race 3 or 4 was homozygous for immunity from race 7. F_3 lines homozygous for susceptibility to race 4 approximated a ratio of 1 semiresistant to 2 heterozygous to 1 susceptible to race 3. Among the F_3 lines immune from race 4, there were approximately 1 resistant to 2 heterozygous to 1 susceptible to race 7. Lines having all plants either immune from or semiresistant to race 3 had all plants either immune from or resistant to race 7. Lines segregating for immunity, semiresistance, and susceptibility to race 3 segregated for immunity, resistance, and susceptibility to race 7. Lines having all plants either immune from or susceptible to race 3 had all plants either immune from or susceptible to race 7.

The results obtained were explained by assuming that immunity from races 7 and 20 in Buda was conditioned by a pair of dominant factors allelic to the pair of dominant factors conditioning immunity from 18 races in J. W. S. The reaction of Buda and the segregation of the F_3 population suggested that Buda carried, in addition to the factors conditioning immunity from race 7, factors conditioning semiresistance to race 3 and resistance to race 7 that were independent of and hypostatic to the immune factors. On this basis the genotype of Buda would be L^1L^1RR and that of J. W. S. L^2L^2rr.

The reaction of Buda to races involved in these tests extended by almost imperceptible steps from immunity to susceptibility. Both the L^1 and the R factors appeared to be operative in conditioning resistance to races to which Buda was resistant. In tests with races to which Buda was semiresistant, moderately susceptible, or susceptible, the factors L^1L^1 had little effect, and reductions in degree of susceptibility appeared to be largely attributable to the R factor. Neither the L^1L^1 nor the RR factors had any appreciable effect on the seedling reaction to races 4, 8, 19, and 22 to which Buda is susceptible.

J. W. S., L^2L^2rr, is susceptible to races 7 and 9, but F_3 plants of genotype L^2L^2RR were resistant to race 7, and their reaction to race 9 varied from moderately susceptible to susceptible.

All plants tested were susceptible to races 16 and 21, to which both Buda and J. W. S. were susceptible.

The R factor was incompletely dominant and, under favorable growing conditions, heterozygous plants of the genotypes L^1L^1Rr or L^2L^2Rr were distinctly less resistant to races producing intermediate infection types, such as races 3 and 7, than homozygous plants L^1L^1RR or L^2L^2RR.

A line having the genotype L^1L^1rr, previously unknown, facilitates the identification of physiologic races differentiated by the semiresistant reaction of Buda.

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A GENETIC STUDY OF COMMON BEAN MOSAIC UNDER CONDITIONS OF NATURAL FIELD TRANSMISSION [1]

By B. L. Wade, *senior geneticist*, and C. F. Andrus, *pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Common bean mosaic (bean virus 1) apparently occurs wherever snap beans (*Phaseolus vulgaris* L.) are grown, but in the southern part of the United States the damage is especially severe. Susceptible varieties may give complete crop failures, and tolerant varieties may develop more pronounced symptoms than they do in northern and western bean-growing areas. Because of the latter fact, it is possible in South Carolina to study the inheritance of reaction to the virus in resistant×tolerant crosses with less difficulty in identifying phenotypes than might be experienced in certain other climatic areas.

In order to obtain a high percentage of virus transmission, most workers with common bean mosaic have employed artificial inoculations. In field plots of the United States Regional Vegetable Breeding Laboratory, Charleston, S. C., natural field spread of common bean mosaic has produced the maximum possible transmission; and, with suitable controls for insuring the identity of the virus, it is possible to rely on field data with a high degree of confidence. The data reported herein were obtained incidentally in a study of bacterial blight resistance in snap beans. The data are submitted because they lead to a different interpretation of the genetics of common bean mosaic from any hitherto recorded.

MATERIALS AND METHODS

The crosses observed in detail were Stringless Black Valentine× U. S. No. 5 Refugee and the reciprocal. U. S. No. 5 Refugee is highly resistant to common bean mosaic [2] as reported from various places. During the last 5 years several thousand plants of this variety have been observed at Charleston, S. C., and no common bean mosaic (bean virus 1) or other virus diseases have been found except in the case of a few obvious rogues. Stringless Black Valentine under early-spring conditions at Charleston, and under normal conditions in the seed-growing areas of the northern and western parts of the United States, ordinarily gives a tolerant reaction to infection with common bean mosaic; i. e., the disease may be present, but the injury is slight or undetectable. Plantings of Stringless Black Valentine made only a few weeks later at Charleston, however, may show much more pronounced symptoms and injury. Other tolerant varieties like Bountiful and U. S. No. 1 Refugee respond much like Stringless Black Valentine.

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[1] Received for publication May 7, 1941. This work was performed chiefly under an allotment from the Special Research Fund authorized by Title I of the Bankhead-Jones Act of June 29, 1935.

[2] Wade, B. L., and Zaumeyer, W. J. U. S. NO. 5 REFUGEE, A NEW MOSAIC-RESISTANT REFUGEE BEAN. U. S. Dept. Agr. Cir. 500, 12 pp., illus. 1938.

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Checks for each generation included the two parents (Stringless Black Valentine and U. S. No. 5 Refugee) and in addition, for the F_1 and F_2 generations, samples of inoculated and uninoculated Stringless Green Refugee. Since Stringless Green Refugee is very susceptible to common bean mosaic, and since mosaic is to some extent a seed-borne disease, a mosaic-free strain was grown under cheesecloth for testing the virus present in Stringless Black Valentine and in the susceptible F_2 segregates. Inoculation of the Stringless Green Refugee in these two cases for the purpose of verifying the presence of the common bean mosaic virus were the only artificial inoculations made.

The F_1 and F_2 plants were grown in rows adjacent to the parents; but in the F_3 generation, as all border rows were U. S. No. 5 Refugee and Stringless Black Valentine, readings were made on the border rows for checks. The borders were about 24 rows apart.

The first observations on common bean mosaic at the Vegetable Breeding Laboratory were made in the spring of 1936. Varieties commonly classified as tolerant showed severe symptoms, and susceptible varieties such as Stringless Green Refugee were so badly injured that only a few seeds per plant were produced. Of 453 U. S. No. 5 Refugee plants observed, none showed any symptoms of mosaic, while in all other varieties noted all plants were infected. The varieties and the number of plants observed were as follows: Stringless Black Valentine, 338; Bountiful, 507; Stringless Green Refugee, 554; and Thousand to One Refugee, 321.

In order to verify the presence and identity of the common bean mosaic virus, seed was harvested from the above-mentioned plants and two series were planted in the greenhouse in the spring of 1937. Sufficient seed was planted for at least 100 plants in each series, and readings were taken on primary or seed-borne mosaic on the first 100 in each case. Except in the case of U. S. No. 5 Refugee, western-grown seed was used as a check. The results from seed from eastern infected plants and from western seed, respectively, were as follows: Stringless Black Valentine from eastern plants infected with seed-borne mosaic, 9.5 percent, and from western seed, 3 percent; Bountiful, 4 percent and 2 percent; Stringless Green Refugee, 25.5 percent and 8.5 percent; Thousand to One Refugee, 17 percent and 29.5 percent; U. S. No. 5 Refugee, 0 percent. From 5 Stringless Black Valentine plants of Charleston origin and 2 of western origin showing primary symptoms, inoculations were made into 10 plants of healthy Stringless Green Refugee. Bountiful and Stringless Green Refugee were also inoculated by using 2 primary infections from the Charleston source and 2 of western origin. In all cases typical symptoms of common bean mosaic were observed. Of 70 Stringless Green Refugee plants inoculated from primary-infected Stringless Black Valentine, 54 showed symptoms, while 10 check plants inoculated from healthy Stringless Black Valentine remained mosaic-free. Of 70 plants of Bountiful, 57 became infected and the 10 checks remained healthy; of 40 plants of Stringless Green Refugee, 32 became infected and the 10 checks remained healthy. The symptoms in all cases were characteristic of Common bean mosaic.

As aphids are usually assumed to be responsible for a large part of the secondary spread of bean mosaic in the field, records have been kept on the aphid population for the last 5 years with readings on approximately May 1, 10, and 20. No attempts have been made to identify the various species found. Ten plants from each of 10 rows taken at random have furnished the basis for the average in each case. The average number of aphids present by May 1 was approximately 1 per plant, whereas by May 10 the number had increased to 2.5, and by May 20 had decreased to about 1.5. Aphids are not ordinarily found in abundance on beans, but it is believed that the numbers found could be responsible for a considerable epidemic of common bean mosaic.

RESULTS

The F_1 generation grown in the spring of 1938 consisted of 9 plants, 3 from the cross Stringless Black Valentine × U. S. No. 5 Refugee and 6 from the reciprocal. These plants all remained free from virus diseases, as did the 37 plants of the U. S. No. 5 Refugee parent; but all 42 of the Stringless Black Valentine parent plants showed symptoms of common bean mosaic. Inoculations made from the Stringless Black Valentine parent to healthy Stringless Green Refugee indicated that common bean mosaic virus alone was present in the Stringless Black Valentine plants (table 1).

In the F_2 generation a ratio of 3 resistant to 1 tolerant was obtained with a total of 179 plants observed, and there was no significant difference in the reciprocals. Chi-square tests indicated satisfactory conformation to a 3:1 ratio. Again the resistant parent remained healthy, while all 106 Stringless Black Valentine plants showed symptoms. Inoculations made from the susceptible F_2 segregates to Stringless Green Refugee showed that the virus responsible for the disease was the common bean mosaic virus (table 1).

TABLE 1. *Results obtained with F_1, F_2, and F_3 progenies from the crosses Stringless Black Valentine × U. S. No. 5 Refugee (A) and reciprocal (B)*

Cross	Classification of plants in F_1 generation		F_2 generation			F_3 generation								
			Classification of plants			Classification of families			Chi square for 1:2:1 ratio in F_3 families[2]	Classification of plants in F_3 segregating families		Chi square for 3:1 ratio for plants in F_3 segregating families		
	Resistant	Tolerant	Resistant	Tolerant	Chi square[1] for 3:1 ratio	Resistant	Segregating	Tolerant		Resistant	Tolerant	Accumulated[3]	Population	Heterogeneity[4]
	No.	*No.*	*No.*	*No.*		*No.*	*No.*	*No.*		*No.*	*No.*			
A	3	0	77	23	0.213	6	15	4	1.340	404	108	13.329	4.167*	9.162
B	6	0	60	19	.038	6	16	5	1.018	496	140	19.746	3.027	16.719
Total	9	0	137	42	.225	12	31	9	2.270	900	248	33.075	7.066**	26.009

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[1] 1 degree of freedom.

[2] 2 degrees of freedom.

[3] Chi square for individual progenies added, with 1 degree of freedom for each family.

[4] Obtained by subtracting population chi square from accumulated chi square. Degrees of freedom, 1 less than number of families.

In the spring of 1939, at the time the F_2 plants previously discussed were grown, some F_3 families from an F_2 plant grown in the fall of 1938 were observed to be segregating in a ratio of 1 resistant to 2 segregating to 1 tolerant. Because of the presence of a considerable amount of bacterial blight an exact count of plants was not made, but in the cross Stringless Black Valentine × U. S. No. 5 Refugee the family ratio was 12 resistant to 23 segregating to 6 tolerant; that of the reciprocal, 3:6:4. None of the chi-square values approached significant deviations, and again there was no indication of differences between reciprocals. Since these observations were preliminary, they are not included in table 1.

The F_3 generation grown in the spring of 1940 consisted of 25 families from the cross and 27 from the reciprocal. The combined data showed 12 resistant, 31 segregating and 9 tolerant families with a nonsignificant chi-square value. Again it was indicated that there was no significant difference in genetic behavior of the cross and its reciprocal.

The F_3 plant population from the 31 segregating families showed a highly significant deficiency of recessives, but heterogeneity chi square showed no difference in the cross and its reciprocal. The F_3 results are shown in detail in table 1. All 104 U. S. No. 5 Refugee check plants were healthy, whereas all 93 Stringless Black Valentine check plants were infected with common bean mosaic virus.

DISCUSSION

In 1921 McRostie[3] reported crosses with various field beans, including Michigan Robust, in which he found resistance to be at least partially recessive. By grouping his data he obtained an F_2 ratio approaching 9 susceptible to 7 resistant. Part of his work was carried out by artificial inoculations, but most of it depended on field spread from susceptible, primarily infected plants.

In 1935 Pierce[4] reported results with various crosses. Robust crossed with Stringless Green Refugee gave a mosaic-susceptible F_1 generation, but Robust crossed with a strain of Great Northern or with Corbett Refugee gave resistant F_1 plants in both cases. Corbett Refugee × Stringless Green Refugee and the reciprocal were resistant in the F_1 generation, and in the F_2 generation they gave 88.8 percent and 82.2 percent resistant, respectively. Idaho Refugee and Wisconsin Refugee behaved in crosses with Stringless Green Refugee about like Corbett Refugee. Pierce did not attempt a genetic explanation of his results.

In 1936 Parker[5] reported differential results from crosses of Stringless Green Refugee × Michigan Robust and the reciprocal, as well as with Corbett Refugee × Stringless Green Refugee. Parker interpreted differences in reciprocals in part on the basis of cytoplasmic inheritance. When Stringless Green Refugee was used as the female parent, some of the results approached a 3:1 ratio of resistant to susceptible.

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[3] McRostie, G P. INHERITANCE OF DISEASE RESISTANCE IN THE COMMON BEAN Jour Amer Soc Agron 13 15-32 1921

[4] Pierce, W. H THE INHERITANCE OF RESISTANCE TO COMMON BEAN MOSAIC IN FIELD AND GARDEN BEANS Phytopathology 25 875-883, illus 1935

[5] Parker, M. C INHERITANCE OF RESISTANCE TO THE COMMON MOSAIC VIRUS IN THE BEAN Jour Agr Res 52 895-915, illus. 1936

In the three genetic studies mentioned the contrasts involved were crosses between resistant and susceptible strains, whereas in the present paper the work is limited to resistant with tolerant. Groupings of resistant, tolerant, and susceptible varieties have been used by many investigators; as, for example, Clark.[6]

Pierce [7] was careful in his work to exclude the possibility of contamination with yellow bean virus (bean virus 2), and both Parker [8] and Pierce used artificial inoculation instead of depending upon field spread. In the work reported herein reliance was placed upon field spread and no artificial inoculations were made except as a check upon the technique and to test for the presence of the common bean mosaic virus. Extensive observations on U. S. No. 5 Refugee, which is susceptible to yellow bean virus, and on other varieties, as well as considerations of the seed-borne nature of the disease observed, indicate that yellow bean virus could not have been present to complicate the results reported herein.

Johnson [9] recently interpreted some symptoms observed on beans from inoculations from apparently healthy *Lathyrus tingitanus* as being due to an allergic response of the bean plants. It seems possible, if allergic responses do occur, that there might be allergic responses of one strain of beans to another and that they might be much more severe with artificial inoculations than with natural insect transmission; consequently artificial inoculations might per se introduce a factor rather disturbing to genetic ratios.

It seems possible that part of the irregularity of ratios encountered by both Pierce and Parker may have come from the presence of a partially lethal variegation in Corbett Refugee and the two strains derived from it, Idaho Refugee and Wisconsin Refugee. U. S. No. 5 Refugee, although related to Corbett Refugee, does not carry the factor or factors for variegation.

SUMMARY AND CONCLUSIONS

In a cross of Stringless Black Valentine × U. S. No. 5 Refugee and the reciprocal, the results with the F_1, F_2, and F_3 generations indicated that the resistance of U. S. No. 5 Refugee to common bean mosaic virus is dominant to the tolerance (which showed a definitely mosaic pattern with marked mosaic symptoms at Charleston, S. C.) of the Stringless Black Valentine, and that a single factor is responsible for the resistance.

There were no significant differences between the cross and its reciprocal.

There was a slight but cumulative deficiency of recessives (22 percent of the population tolerant, theoretical 25 percent), which reached significance in the total populations from segregating families in F_3.

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[6] CLARK, N., compiler. MOSAIC RESISTANT CANNING BEAN SHOWS PROMISE. Wis Agr Exp Sta Bul 421 60–62, illus 1931

[7] See footnote 4

[8] See footnote 5

[9] JOHNSON, JAMES INOCULATION OF BEAN WITH EXTRACTS FROM OTHER HEALTHY LEGUME SPECIES (Abstract) Phytopathology 30 12 1940.

GROWTH SUBSTANCE IN CROWN GALL AS RELATED TO TIME AFTER INOCULATION, CRITICAL TEMPERATURE, AND DIFFUSION [1]

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INTRODUCTION

The importance of growth substances in the normal development of plant tissues has suggested that, when present in unusual amounts, they may be more or less directly responsible for atypical and pathological growth. Attention has been directed especially to such growths as crown gall caused by *Phytomonas tumefaciens* (Smith and Town.) Bergey et al. Beta-indole-acetic acid and substances related in effect, like auxin-a and auxin-b in particular, have been considered the chemical means by which the bacteria induced this diseased overgrowth.

The evidence for this has been presented and discussed by various workers. For example, beta-indole-acetic acid has been suggested with various degrees of emphasis by Němec (*11*), Brown, and Gardner (*2*), Link, Wilcox, and Link (*5*), and Berthelot and Amoureux (*1*) as the responsible stimulating agent produced by the bacteria. Locke, Riker, and Duggar (*6*) described symptoms on plants bearing crown gall as similar in several particulars to those induced by beta-indole-acetic acid. Limited trials by diffusion technique indicated that more growth substance is present in recently inoculated tomato stems than in control stems. However, trials with older galls and with extractions failed to show differences between gall and control tissue. In addition, Locke, Riker, and Duggar (*7, 8*) found that crown gall and related avirulent bacteria in peptone broth produced in similar amounts a substance acting like beta-indole-acetic acid in its sensitivity to acid and alkali, and that both healthy and diseased tomato tissue contained material likewise similar to this acid. Kraus, Brown, and Hamner (*4*) demonstrated the similarity in structure between galls induced by *Phytomonas tumefaciens* and beta-indole-acetic acid.

The present investigations have been aimed at quantitative determinations of certain growth substances (1) that are found at progressive intervals following crown gall inoculation, (2) that are present in tomato stems grown both above and below the critical maximum temperature for crown gall formation, and (3) that diffuse from healthy and diseased tissue. A preliminary statement of some of this work has already appeared (*13*). With such studies it seemed possible to clarify the relation, if any, of growth substances such as beta-indole-acetic acid to the pathogenicity of crown gall bacteria.

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[2] Italic numbers in parenthesis refer to Literature Cited, p. 405.

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MATERIALS AND METHODS

The tomato (*Lycopersicum esculentum* Mill.) was used as a convenient host. Plants were grown individually in 4-inch pots in the greenhouse at approximately 24° C. and inoculated by stem punctures when they were about 8 inches tall. Galls were conspicuous in about 10 days and equal to the diameter of the stem in 3 to 4 weeks. Suitable control punctures were always made in a separate series of similar plants.

The growth substance was extracted by a slight modification of the method of Van Overbeek (*16*). A 25-gm. sample of fresh plant tissue was cut into pieces about 2 mm. in thickness and placed in a 500-ml. Erlenmeyer flask. About 250 ml. of freshly distilled peroxide-free ether was added immediately and the whole placed overnight at 4° C. The following morning the ether was separated from the plant material and the water. The extract-containing ether was heated in a distillation apparatus on a steam table until only about 2 ml. remained. The distilling flask was cooled under a cold water tap and the ether poured into a 10-ml. vial. One ml. of distilled water was pipetted into the vial, which was then placed on the steam table until the remaining ether had evaporated. Thus the ether extract of 25 gm. of plant tissue was contained in 1 ml. of water. This 1 ml. of water was mixed with an equal volume of 3-percent agar, and the mixture was tested for the presence of growth substance. Tests were always made on the same day that the extracts were prepared. The galls and control tissue were treated identically in extraction. While it is recognized that the ether extraction may not remove all of the free growth substance present, it appears that the relative amounts obtained from the control tissue and the crown gall tissue should be comparable.

Considerably higher yields of growth substance have been secured with other methods from plant tissues by various workers, including Avery, Berger, and Shalucha.[3] Apparently these higher yields are due largely to hydrolysis of precursors rather than to improved extraction of free auxin. The method used seems one of the best at present available for securing free auxin.

The standard *Avena* test (*18*) was used, with slight modifications as explained by Locke, Riker, and Duggar (*7*), in determining the amounts of growth substances present. It is apparent that the growth substances measured were those acting upon *Avena* coleoptiles under the conditions of experimentation. Obviously, various growth substances may have been present which were not detected by this technique.

The measurements secured are based upon averages of curvatures of 24 to 36 coleoptiles. Thus, the data included in this paper, together with those from various preliminary trials not mentioned because of the space they would occupy, are based upon about 7,000 coleoptile measurements.

The critical-temperature studies were made in two cubical chambers 4 feet square, each illuminated by ten 30-inch 30-watt daylight fluorescent lamps. The plants received light for 12 to 15 hours each day. The temperature was thermostatically maintained at 27° C. in one chamber, where galls were formed, and at 31° in the other, where no galls developed. This is below and above, respectively, the maximum temperature for gall formation determined by Riker (*12*) and confirmed by the present studies.

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[3] AVERY, GEORGE S., BERGER, JULIUS, and SHALUCHA, BARBARA. THE TOTAL EXTRACTION OF FREE AUXIN AND AUXIN PRECURSOR FROM PLANT TISSUE. Amer. Jour. Bot. 28: 596-607, illus., 1941.

EXPERIMENTAL RESULTS

COMPARISON OF RESULTS IN TERMS OF COLEOPTILE CURVATURE AND OF HETEROAUXIN EQUIVALENTS

The results of the *Avena* test are commonly reported in the average amount of curvature produced. This seems subject in certain localities to the error of variations from day to day in sensitivity of the test plants. Some variations also occur within a given day. In these studies results were taken on known material several times within a day, as well as on different days, and a comparison was made of the variations secured. The results showing "within-a-day" and "day-to-day" variations are given in table 1, and an analysis of their variance (*15*) in table 2.

The variation between days has an *F* value of 10.96 that greatly exceeds the appropriate 5-percent point (2.15); so the existence of a day-to-day variation can be inferred. Consequently, the data presented later are corrected to heteroauxin equivalents in the interest of accuracy.

TABLE 1. *Variation in Avena coleoptile curvature, with a concentration of 100 gammas per liter of beta-indole-acetic acid, compared within the same day and on different days, 1940*

Date	Average negative curvatures for replication 1	2	3	Totals
	Degrees	*Degrees*	*Degrees*	*Degrees*
Feb 20	11	12	11	34
Mar 3	9	7	9	25
Mar 5	11	8	7	26
Mar 10	9	8	7	24
Mar 26	10	7	12	29
Mar 27	8	8	7	23
Mar 31	15	14	13	42
Apr 7	11	10	12	33
Apr 10	14	15	16	45
Apr 14	8	6	8	22
Apr 28	8	7	7	22
May 12	10	7	9	26
May 19	11	9	10	30
Total	135	118	128	381

TABLE 2. *Analysis of variance on data from table 1*

Source of variance	Degrees of freedom	Sums of squares	Mean square	*F*	*F* at the 5 percent point
Between days	12	219.59	18.30	10.96	2.15
Within days	26	43.33	1.67		
Total	38	262.92			

PROCEDURE WITH ANALYSIS OF VARIANCE

The statistical procedure employed with the analysis of variance in table 2 is briefly given. Denoting by σ^2 the variance of determinations made on a single day, and by σ_d^2 the variance due to day-to-day variations (the actual days involved being regarded as a random sample from a "population" of days), one can estimate these quantities as follows:

$$\text{Est. } \sigma^2 = \text{Within days mean square} = 1.67$$

$$\text{Est. } \sigma_d^2 = \frac{(\text{Between days mean square}) - (\text{Within days mean square})}{3} = 5.54$$

where the divisor 3 represents the three determinations made each day.

The variance of the mean, $\bar{x}$, of the three determinations on a random day is

$$\sigma_{\bar{x}}^2 = \sigma_d^2 + \frac{\sigma^2}{3}$$

where σ_d^2 represents the variation arising from drawing a random day and $\frac{\sigma^2}{3}$ represents the inaccuracy arising from the fact that sensitivity for that day is estimated by only three determinations. In the day-to-day variation, σ_d^2 represents the variation which would still be present if an infinite number of determinations were made each day—that is, if the time value for the day were determined exactly—so $\sigma_{\bar{x}}^2$ will always exceed σ_d^2 no matter how accurately one determines the sensitivity prevailing on particular days. The "error" in determining the value for a particular day is always of the form $\frac{\sigma^2}{n}$ and can be reduced by increasing n (the number of replicates).

Substitution of the estimated values of σ^2 and σ_d^2 yields $\sigma_{\bar{x}}^2 = 5.54 + 0.56$; so day-to-day variation constitutes about 90 percent of the error in a mean of three determinations made on a single day.

A correction for the day-to-day variation secured seemed desirable.

CALCULATION OF HETEROAUXIN EQUIVALENTS

A correction based on the activity of a known substance is called for because many factors, beyond the practical control of the investigators, may influence the sensitivity of the coleoptiles from day to day and from laboratory to laboratory, but their consideration is beyond the scope of this paper. This correction necessitated the use of a known concentration (100 gammas per liter) of beta-indole-acetic acid with each assay of the unknown materials. The formula used in making conversions to the heteroauxin equivalents, shown at the right of succeeding tables, was adapted from that of Van Overbeek (*16*). It is as follows:

$$\frac{C \times I \times V}{W} = \text{gamma heteroauxin equivalents per liter of water in the tissue}$$

where C is the coleoptile curvature, in degrees, produced by the plant extract; I is the concentration of heteroauxin, in gammas per liter, required to give a curvature of 1 degree; V is the volume in milliliters of agar and water in which the extract was taken up; and W is the water content, in grams of plant tissue extracted.

COMPARISON OF INOCULATED AND CONTROL TISSUE AT SUCCESSIVE TIME INTERVALS AFTER INOCULATION

Measurements of growth substances have been made to clarify the suggestions about their importance in gall formation. Half of a series of tomato plants were puncture-inoculated with crown gall bacteria, and the others were given control punctures. It was considered that if auxin (i. e., any of the growth substances causing negative *Avena* curvature) was responsible for crown gall development, differences in auxin content might appear between gall and control tissue. Since the auxin content might fluctuate in relation to the progress of gall development, examinations were made at about 1, 4, 8, and 16 days after inoculation. On the last day proliferation was well under way.

The auxin in the growing tip was removed from the complex in three series by using only decapitated plants. In three other parallel series whole plants were used. The work was done in the early part of 1939.

As representative of the work done, detailed results of the series inoculated February 18 are given in the upper part of table 3.

TABLE 3.—*Crown gall and control tissue compared at progressive intervals in regard to amounts of growth substances that produce Avena coleoptile curvatures and their heteroauxin equivalents, 1939*

Plant series	Time after treatment	Average negative curvature[1]			Heteroauxin equivalent[1]	
		Inoculation	Puncture control	Heteroauxin control[2]	Inoculation	Puncture control
	Days	*Degrees*	*Degrees*	*Degrees*	*Gammas per liter*	*Gammas per liter*
Decapitated plants:						
Results in series inoculated Feb. 18	1	5	4	9	5	4
	4	12	12	11	10	10
	8	7	6	11	6	5
	16	2	3	9	2	3
Averages of results in 3 series inoculated Feb. 18, Feb. 22, and Mar. 8	1	6	5	9	6	5
	4	9	8	12	7	6
	8-9	6	6	10	5	5
	16-17	6	5	9	5	5
Whole plants:						
Averages of results in 3 series inoculated Apr. 5, Apr. 19, and May 3	1	9	10	8	10	10
	4	12	11	10	11	10
	8	10	8	8	12	9
	15-18	10	10	9	10	10
Averages of all trials	1	7.5	7.2	8.7	7.7	7.4
	4	10.8	9.8	11.0	8.8	7.9
	8-9	8.1	7.3	9.2	7.8	7.0
	15-18	8.4	8.1	9.2	8.1	7.8
Averages of all trials corrected on basis of total nitrogen content	1				7.0	7.4
	4				8.0	7.9
	8-9				7.1	7.0
	15-18				7.4	7.8

[1] Expressed in nearest whole number except averages of all trials.

[2] A concentration of 100 gammas per liter was used.

An example of the conversion to heteroauxin equivalents is given with the first calculation in table 3, where 5 degrees of curvature are equivalent to 5 heteroauxin equivalents. The degrees curvature are given in the table in terms of the nearest whole number, namely, 5, although the actual figure in this case was 4.7, this being the average curvature in degrees of 24 coleoptiles. Thus, C is 4.7; I is 100 gammas per liter divided by 9 degrees of curvature or 11.1; and V is 1 ml. of agar plus 1 ml. of material, or 2. Since greenhouse galls contain about

10.3 percent of dry matter (*10*) in 25 gm. of tissue, there are 2.6 gm. of dry matter and 22.4 gm. of water; thus *W* is 22.4. (Since there seems to be no significant difference between the percentage of dry matter in greenhouse galls and control stems, the same correction factor is used here for both gall and control tissue.) In this case the heteroauxin equivalents are about 4.6 gammas per liter of water in the plant tissue. The nearest whole number is 5, which appears in table 3. Detailed results of other trials are omitted because of their volume.

The results with decapitated plants in the series inoculated February 18, February 22, and March 8, respectively, are summarized in table 3. For making these averages the daily determinations to one decimal point were used. There is an average variation from 5 to 7 gammas of heteroauxin equivalents per liter of liquid in gall tissue and from 5 to 6 in that of puncture controls.

Whole plants were used in three series, also summarized in table 3. Except for higher auxin values throughout, the results are similar to those with the decapitated plants. Since the growing tip is a ready source of auxin, the greater amount found was anticipated.

The galls developed well in decapitated plants in which only half as much of auxin was detected as in whole plants. This in itself indicates that gall development is independent of auxin change from 5 to 10 gammas per liter.

The summary of all the tests is given in table 3. The greater amount of growth substance on the fourth day after inoculation does not seem significant. It appears that there is a slight increase in the amount of growth substance in the inoculated tissue at each average time interval over that in the control puncture. When one recalls that 8 gammas per liter represents 8 parts in 1,000,000,000, one recognizes that the difference between 8.8 and 7.9 is hardly significant. Even this difference disappears when a suitable correction is added.

The living protoplasm is reasonably considered to be the portion of the tissue active in the production of growth substances. So a comparison of gall and control tissue on a total nitrogen basis may be a closer approach to an estimation of the amount of living substance than is a comparison on a weight basis. Nagy et al. (*10*) showed that in greenhouse galls total nitrogen was 3.3 percent of the dry matter, while in corresponding stems it was 3.0 percent. A comparison of auxin values on a total nitrogen basis is doubtfully applicable where plants have been recently inoculated since there is then only a slight development of gall tissue. However, when such corrections are applied to the averages of all these trials, the results are as shown at the bottom of table 3. Here any indication of a significant difference between gall tissue and control tissue seems to disappear.

COMPARISON OF TISSUES GROWN ABOVE AND BELOW CRITICAL TEMPERATURES FOR GALL FORMATION

The auxin content of tomato tissue grown at 27° C., where galls develop, and at 31°, where no galls develop (*12*), was studied early in 1940. Experimental plants were puncture-inoculated at 1- to 2-inch intervals in several internodes. They were grown in two cubical chambers about 4 feet on a side located in the basement of the greenhouse. With rare exceptions there was a variation from the set temperatures (27° and 31°) of less than 1°. The plants in each chamber were illuminated from above for 12 to 15 hours by 10 daylight fluorescent, 30-inch, 30-watt lamps with aluminum reflectors about 30 inches above the pots or boxes. No effort was made to regulate the humidity of the air, which fluctuated in both chambers around 65 percent of saturation. Fresh air was supplied by forced ventilation. The plants were harvested 4 to 5 weeks after treatment.

Fifteen-gram samples of gall tissue, and of stem tissue from between the galls, were taken from plants grown at 27° C. A similar sample of stem tissue was taken from between the inoculation points on plants grown at 31°. The tissues were ether-extracted as described earlier and the extracts assayed on *Avena* coleoptiles.

The results from temperature studies are shown in table 4, which is arranged in a manner similar to table 3. In general, the amount of growth substance found was similar to that in whole plants, as recorded in table 3. The difference in the amounts of growth substances secured from the stems at 27° and 31° C. is not considered significant as far as gall formation is concerned. It is much less than that between the whole and decapitated plants (table 3), and even in the latter instance gall development was not significantly influenced. Whatever difference exists in the temperature series can probably be attributed to less vigorous growth at 31° than at 27°. Corrected on a total-nitrogen basis the averages show approximately 2 gammas per liter more of growth substances in the galls than in the controls. This is not considered significant and possibly can be explained by the more active growth of the gall tissue than the adjacent stem tissue of the same plant.

TABLE 4.—*Crown gall and control tissue compared at 27° and 31° C in regard to the amounts of growth substances that produce Avena coleoptile curvature and their heteroauxin equivalents, 1940*

Date	Average negative curvature [1]				Heteroauxin equivalents		
	Gall at 27° C	Stem at 27° C	Stem at 31° C	Heteroauxin control [2]	Gall at 27° C	Stem at 27° C	Stem at 31° C
	Degrees	*Degrees*	*Degrees*	*Degrees*	*Gammas per liter*	*Gammas per liter*	*Gammas per liter*
Jan 15	23	14	13	19	19	12	10
Mar 16	15	13	16	15	16	14	17
Apr 2	14	12	10	13	17	14	12
Apr 26	21	12		17	20	11	
May 28	12	12	10	16	11	11	9
Averages of all trials	17.2	12.8	12.3	15.8	16.3	12.1	11.6
Averages corrected on total nitrogen basis					14.8	12.1	11.6

[1] Expressed in nearest whole number except for averages.
[2] A concentration of 100 gammas per liter.

Although no significant differences were found in the amount of growth substances either in crown gall and control tomato tissue or in tomato tissues grown above and below the critical temperatures, there are two remaining possibilities. More auxin might have been produced in the gall than in control tissue, but it might have disappeared rapidly because (1) it was decomposed during gall formation, or (2) it was diffused away so rapidly that it failed to accumulate. Evidence about the first is difficult to secure. The data from decapitated and whole plants in table 3 seem not to support this possibility. The second possibility was studied by means of diffusion technique.

DIFFUSION FROM GALL AND CONTROL TISSUE

The amount of growth substance which diffuses from crown gall and control tissue was studied by a technique similar to that employed by Went (*17*) with the tips of *Avena* coleoptiles. Tomato plants, from which gall and control tissues were obtained, were grown in the greenhouse and inoculated under conditions analogous to those in the extraction experiments. The materials were tested for diffusible growth substances at 1, 4, 8, and 16 days after inoculation.

Two stem segments with a combined weight of 500 mg. and with one inoculation point each were cut out with a razor blade. In several experiments no growth substances were obtained by diffusion from crown gall tissue itself. For this reason, about 2 mm. of stem tissue was left below the inoculation point, or gall tissue, when the inoculated segments were removed from the plant. The lower end of each section was thoroughly washed with a "salt solution" composed of NaCl, 6 gm.; KCl, 0.4 gm.; $CaCl_2$, 0.4 gm.; and H_2O, 1,000 ml. (*3*). This removed the exudate from the cut cells and supplied a moisture film to facilitate diffusion. The stem segments were then placed upright on agar blocks measuring 1 by 8 by 11 mm. and consisting of 1½ percent agar in the salt solution. The blocks were on glass microscope slides. The slides with their corresponding agar blocks and stem segments were placed in Petri dishes lined with wet filter paper for 2 hours at laboratory temperature. Following this diffusion the agar blocks were each cut into 12 equal parts and applied to *Avena* coleoptiles. Similar treatment was given tissue with control punctures. The results are correspondingly based on the average curvature of 24 coleoptiles for each material per test.

The polarity of growth substance movement was apparent since no active substance was obtained in trials when stem segments were placed top down on the agar blocks.

TABLE 5.—*Crown gall and control tissue compared at progressive intervals in relation to the diffusion of growth substance, 1939*

Trial	Time after treatment	Average negative curvature[1]			Heteroauxin equivalents[1]	
		Inoculation	Puncture control	Heteroauxin control[2]	Inoculation	Puncture control
	Days	*Degrees*	*Degrees*	*Degrees*	*Gammas per liter*	*Gammas per liter*
Trial of Oct. 8	1	14	15	17	33	36
	4	9	2	12	29	8
	8	11	8	12	35	27
	16	9	10	9	39	40
Averages of 4 trials	1	9.5	10.5	12.2	30.5	33.7
	4	10.4	7.0	16.5	24.7	16.6
	8	9.9	7.5	15.1	25.7	19.5
	16	7.5	7.9	8.7	33.8	35.6
Averages corrected on total nitrogen basis	1				27.7	33.7
	4				22.5	16.6
	8				23.4	19.5
	16				30.7	35.6

[1] In nearest whole number except for averages.

[2] A concentration of 100 gammas per liter.

Numerous preliminary experiments were conducted to determine the relation of coleoptile curvature to (1) the area of the diffusion surface, (2) the diffusion time, and (3) the wet weight of the sample. The results were quite variable. Within the limits of the studies no significant relation was determined between these factors and the coleoptile curvatures. The details of results are omitted. Since inoculated and control tissues were similar respecting these factors, the results given in table 5 are primarily comparative. The values, while within the supposed range of quantitative coleoptile sensitivity, contain objectionable variations. One representative trial appears in table 5. The average of 4 trials is given with corrections on the total-nitrogen basis.

As in previous trials, there was no significant difference between the amount of growth substance secured from inoculated stems and from those receiving only control punctures. Thus, it appears that the diffusion of growth substances from crown gall is not significantly higher than that from control tissue.

DISCUSSION

The mechanism by which the crown gall bacteria induce the characteristic pathological cell growth has been investigated by a number of research workers, as explained in the introduction. Recently the chemical cause of this diseased growth has been suggested or claimed to be beta-indole-acetic acid or some closely related substance elaborated by the bacteria during growth in the host tissue. This concept has been based upon several lines of evidence: (1) The crown gall bacteria produce beta-indole-acetic acid or a similar substance in suitable cultures; (2) extracts of the crown gall culture and of crown gall tissue when applied to suitable plants have induced proliferation more or less closely resembling crown gall tissue; and (3) preparations of beta-indole-acetic acid when applied to suitable tissue have also induced proliferation closely resembling crown gall.

From these lines of evidence it has appeared to some that beta-indole-acetic acid is the active bacterial metabolite and that the crown gall problem has been solved. All of these different lines have been studied and confirmed in the writers' laboratories. However, certain additional experiments have been made in parallel with these investigations. They have shed further light upon the problem and cast doubt upon the validity of certain conclusions.

The necessity for rigid controls on experiments dealing with the mechanism of cell stimulation in crown gall and some suggestions regarding them have been indicated by Riker and Berge (*14*). When such controls were applied to the present investigation, it appeared that not only the crown gall organism but various nonpathogenic bacteria produced beta-indole-acetic acid or closely related growth substances in suitable culture media. On the most favorable medium for this production the maximum amount of growth substances ever secured was about 125 gammas per liter (*7*).

A variety of extracts from plants and also various chemicals are capable of inducing proliferations in plants. Scores of such substances have been listed in the review by Riker and Berge (*14*). The overgrowths induced by beta-indole-acetic acid are histologically similar in many respects to those induced by crown gall bacteria (*4*). Although 1 percent and sometimes smaller amounts may be effective on some plants, the common dosage is 3 percent in lanolin, which corresponds to 30,000,000 gammas per liter. When used for inducing roots in cuttings, aqueous solutions of beta-indole-acetic acid are commonly diluted to less than 1 in 20,000 (50,000 gammas per liter). The concentrations occurring, therefore, in bacterial cultures and in plant tissues are hundreds or thousands of times smaller than those employed for inducing pathological growth with chemicals.

The "host range" of beta-indole-acetic acid is somewhat different (*13*) from that of crown gall bacteria. There are some plants that respond to this substance that are not susceptible to crown gall, while there are others susceptible to crown gall that do not respond to beta-indole-acetic acid.

Thus, it appears from the evidence presented here (1) that the present writers have confirmed the experimental results (mentioned earlier) of other investigators, (2) that they have provided new evidence shedding further light upon the interpretations, and (3) that these interpretations need revision. So far as the present writers are aware, there is no satisfactory evidence showing that the pathogenicity of crown gall bacteria can be attributed to the production of heteroauxin or similar growth substances measured by the technique here employed.

However, the crown gall bacteria produce various growth substances other than auxin use, in fact, everything they require for growth in a purely synthetic medium. Measurements reported by McIntire, Riker, and Peterson (*9*) have shown the synthesis by this organism of unusually large quantities of biotin, large amounts of flavin, and moderate amounts of thiamin and pantothenic acid. This ability of the crown gall organism to manufacture other growth substances needs further study in relation to its cell-stimulating property.

SUMMARY

Growth substances of the heteroauxin group have been studied in relation to crown gall formation.

About 90 percent of the variations in the *Avena* coleoptile test as used were eliminated by taking the results in terms of heteroauxin equivalents.

No significant differences in auxin content were found between inoculated and control tissue 1, 4, 8, and 16 days after inoculation, especially when comparisons were made on a total nitrogen basis.

Galls and control stems from decapitated tomatoes contained approximately half as much auxin as those from whole plants. However, galls were similar in size on both kinds of plants.

No significant difference was found between the auxin content of tomato stems grown at 27° C., where galls develop, and at 31°, where galls do not develop.

No significant difference was found in the amount of auxin diffusing from stems bearing galls and from control stems.

The experiments of certain other investigators, from which suggestions or claims were made that substances like heteroauxin were responsible for the pathogenicity of crown gall bacteria, have been repeated. Although their results have been generally confirmed, their conclusions have appeared unjustified. So far as the writers are aware, the crown gall bacteria are pathogenic independently of auxin production.

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A STUDY OF SAMPLING IN CROSS-SECTION MEASUREMENTS OF WOOL FIBER[1]

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INTRODUCTION

Since the present wool-grading system is based largely on fiber diameter, standardization of the technique of sampling and measurement is of great importance to the industry. Various procedures are now employed for the measurement of wool-fiber diameter, including the cross-section method, which is recognized as an official method by the American Society for Testing Materials (*1, pp. 173 176*).[3]

In the previous paper (*2*) a discussion of methods which have been and are now in use in the United States and foreign countries was presented. It was pointed out that in the case of the conventional rapid cross-section method it was not possible to record the diameter at more than a limited number of points along the fiber length. This is in contrast to the method employed by the British Wool Industries Research Association and approved by the International Wool Textile Organization (*10*) in which the fibers are cut into very short lengths in order that the entire length of the fiber may be represented in the final sample measured.

Malan, Carter, and Van Wyk (*7*) have recently reported a study of various methods of sampling Merino wool fiber for diameter measurements. One set of fibers was cut transversely into as fine fragments as possible throughout the length of the fiber; the second set was cut transversely at three points (base, middle, and tip) along the fiber lengths; the third was cut transversely at the middle of the staple; and the fourth obliquely across the base and tip of two different sections of fiber. Euparal was used as the mounting medium and the diameters of the fibers as they fell onto the slide were measured microscopically by two different observers, the assumption being that the fibers will fall in such a manner that a large number of measurements will form an even distribution between greatest and least diameters represented in the sample. From a statistical standpoint the authors concluded that the third and fourth methods had much to recommend them, the fourth method being the most difficult sampling procedure. Significant differences between the measurements of the two observers were found. It was concluded that the measurement of 250 fibers on a single well-prepared slide is sufficient.

The present study was undertaken to determine the variation in fiber diameter and contour in samples of fiber from three positions

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[3] Italic numbers in parentheses refer to Literature Cited, p. 415.

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along the fiber length. It was hoped that the statistical analysis would indicate whether, in grading flocks of sheep, there is an advantage in measuring fiber diameter at more than one point along the fiber length and also whether, in the grading of individual samples of wool fiber, a significant difference in diameter and contour ratio exists at three specified points along the fiber length. The animals represented in this study were fed the same diet and maintained under the same environmental conditions.

METHODS AND MATERIALS

Samples of wool fiber from the Notail breed of sheep raised at the South Dakota Agricultural Experiment Station (*11*) were taken from the shoulder, middle, and thigh portions of nine individuals. Portions of the samples were then cut into three equal parts, and the fiber thoroughly cleaned in carbon tetrachloride, stained with a saturated solution of picric acid, and stored in a desiccator containing sulfuric acid of a concentration calculated to maintain an atmosphere of approximately 65 percent relative humidity. Approximately one-half of each sample portion was required to prepare the two slides for fiber measurements. The procedures used to prepare the microscopic slides and to secure the fiber diameter measurements of 200 fibers per sample have been described previously (*2*). In each instance the diameter reported is that found at approximately the middle of each sample portion of the fiber, but it is not possible in the cross-section method to record fiber diameter for each individual fiber used at the three points measured. All the measurements were made by a single observer.

The mean diameter and contour ratios were determined, and the standard deviation was calculated to furnish a measure of the variability. The formula suggested by Snedecor (*9*) has been used throughout in the chi-square test for homogeneity of errors, and in certain instances Bartlett's test for homogeneity (*4*, *8*) was applied. The analysis of variance developed by Fisher (*6*) has been applied throughout the study.

EXPERIMENTAL RESULTS

It is recognized in the analyses of the data used in this discussion that the nine fleeces employed do not necessarily characterize the Notail breed. These particular sheep were chosen for the study because it was believed that they exhibited certain superior fiber qualities and would be desirable animals to use for breeding purposes. It was felt, however, that the data obtained would be useful in suggesting certain fiber relationships that may exist in a flock of sheep and the advantages or disadvantages of the method used to study fiber diameter and contour. The latter information would be of particular value to the wool grader.

Location on the animal, such as shoulder, middle, and thigh, will be indicated by that designation, and the particular point on the fiber will be referred to as position in the fiber.

In table 1 are given the average diameters of 200 fibers from each of 3 locations on each of 9 sheep at 3 points along the fiber length. The standard deviation of single observations has been calculated to indicate the variability of these measurements.

TABLE 1 — *Average diameter and standard deviation of single measurements of 200 wool fibers from each of 3 positions in the wool fiber at 3 locations on the Notail breed of sheep* [1]

Location	Sample No	Flesh: Mean diameter	Flesh: Standard deviation	Midpoint: Mean diameter	Midpoint: Standard deviation	Tip: Mean diameter	Tip: Standard deviation	Average: Mean diameter	Average: Standard deviation
		μ	μ	μ	μ	μ	μ	μ	μ
Shoulder	4190	31.58	6.10	31.82	5.66	30.35	5.60	31.25	5.79
	4630	28.40	4.64	30.36	4.94	31.42	5.01	30.06	4.86
	4671	26.02	6.13	27.28	5.88	26.77	5.25	26.89	5.77
	4813	26.77	3.98	27.84	3.56	28.61	3.29	27.74	3.62
	4901	23.46	4.25	24.86	4.23	24.38	4.20	24.23	4.23
	4914	29.12	4.65	29.33	4.81	28.88	4.65	29.11	4.70
	5151	25.32	4.09	26.10	4.51	23.74	4.04	25.05	4.22
	5304	28.98	4.80	30.52	5.28	30.06	4.91	29.85	5.00
	5412	24.79	4.46	24.85	4.93	25.30	4.16	24.98	4.53
	Average	27.23	4.84	28.11	4.91	27.72	4.61	27.68	4.70
Middle	4190	30.23	6.55	31.77	5.70	30.67	5.46	30.89	5.92
	4630	27.05	5.46	28.96	5.84	30.60	5.71	28.87	5.67
	4671	28.68	4.17	29.12	4.11	29.12	4.02	28.97	4.10
	4813	27.17	6.09	26.83	5.55	28.48	5.46	27.49	5.71
	4901	23.74	5.21	26.23	5.01	23.80	5.22	24.59	4.84
	4914	28.56	5.25	28.72	5.05	28.06	5.20	28.45	4.83
	5151	25.94	5.24	27.37	5.39	28.34	4.48	27.22	5.05
	5304	26.51	4.70	28.22	4.50	27.94	4.13	27.56	4.45
	5412	24.81	4.93	24.14	4.62	25.22	4.58	25.39	4.71
	Average	26.96	5.33	27.93	5.12	28.02	4.95	27.71	5.06
Thigh	4190	29.21	6.91	31.37	5.99	29.18	5.57	29.93	6.18
	4630	28.22	10.25	31.58	11.17	36.62	10.61	32.14	10.68
	4671	27.61	10.11	25.98	7.87	27.23	8.68	26.94	8.93
	4813	32.82	6.37	35.00	6.82	35.88	5.58	34.57	6.28
	4901	27.17	8.33	27.40	8.10	27.78	8.50	27.45	8.31
	4914	30.28	7.67	30.41	6.44	31.90	7.22	30.87	7.13
	5151	27.80	7.34	28.06	6.90	30.22	6.71	28.69	6.99
	5304	34.90	8.86	34.38	7.87	33.82	7.89	34.37	8.22
	5412	30.84	10.30	27.32	6.88	30.88	6.83	29.68	8.17
	Average	29.88	8.58	30.17	7.70	31.50	7.66	30.52	7.99

[1] All average standard deviations in this table were obtained as an average within fleeces by averaging the squared standard deviations and extracting the square root

If a composite is made of the measurements of the fiber in the three positions, considerable variation will be found to exist in fiber diameter between sheep-within-fiber-position at any given location (table 1). On an average the standard deviation of the fiber at the thigh of the fleeces was greater than at the middle and shoulder The middle and shoulder showed approximately the same variability in standard deviation among the three portions of fiber within each location

As shown in table 2, with few exceptions the ratio of the greatest diameter of the fiber to the diameter at right angles to it was greater than the 1.20:1 ratio reported by Barker (*3*) for the ideal fiber. With the exception of 1 middle and 11 thigh samples all contour ratios were between ratios of 1.2:1 and 1.3 to 1, the average ratio for wool fiber computed by Von Bergen and Krauss (*5*). At each position in the fiber the average contour ratios increased progressively from the shoulder to the middle to the thigh locations on the fleeces, and the standard deviations showed the same tendency

The chi-square test for homogeneity of errors has been used to determine whether the variability of the fiber diameter and contour measurements for separate locations on the fleeces and among the various fiber positions were homogeneous (*9*). The Bartlett test was applied in all the diameter measurements at the shoulder location on the fleeces, and in no case did the values obtained change the significance of the errors. Therefore, the results with Snedecor's method are used throughout the present discussion.

TABLE 2.—*Average contour ratios and standard deviations of single measurements of 200 wool fibers from each of 3 positions in the wool fiber at 3 locations on the Notail breed of sheep*[1]

Location	Sample No.	Flesh: Mean fiber contour ratio	Flesh: Standard deviation	Midpoint: Mean fiber contour ratio	Midpoint: Standard deviation	Tip: Mean fiber contour ratio	Tip: Standard deviation	Average: Mean fiber contour ratio	Average: Standard deviation
Shoulder	4190	1.24	0.141	1.29	0.195	1.25	0.160	1.26	0.167
	4630	1.21	.114	1.21	.112	1.25	.130	1.22	.119
	4671	1.25	.142	1.24	.146	1.26	.155	1.25	.148
	4813	1.24	.133	1.22	.139	1.25	.153	1.24	.142
	4901	1.26	.150	1.27	.149	1.27	.182	1.27	.160
	4914	1.22	.133	1.28	.143	1.25	.157	1.25	.145
	5151	1.24	.146	1.24	.153	1.24	.158	1.24	.152
	5304	1.22	.126	1.23	.136	1.28	.146	1.24	.136
	5412	1.20	.127	1.23	.167	1.23	.123	1.22	.140
	Average	1.23	.135	1.24	.150	1.25	.152	1.24	.146
Middle	4190	1.25	.145	1.29	.162	1.28	.163	1.27	.157
	4630	1.23	.132	1.25	.148	[illegible]	.151	1.24	.144
	4671	1.30	.181	1.28	.183	1.27	.166	1.28	.177
	4813	1.25	.151	1.27	.142	1.27	.163	1.26	.152
	4901	1.25	.139	1.31	.169	1.26	.154	1.27	.154
	4914	1.27	.150	1.27	.149	1.28	.158	1.27	.152
	5151	1.24	.144	1.28	.172	1.30	.201	1.27	.175
	5304	1.22	.150	1.21	.117	1.26	.142	1.23	.137
	5412	1.23	.148	1.24	.140	1.25	[illegible]	1.24	.148
	Average	1.25	.149	1.27	.155	1.27	.163	1.26	.156
Thigh	4190	1.30	.165	1.31	.190	1.28	.155	1.30	.171
	4630	1.26	.186	1.29	.163	1.33	.196	1.29	.182
	4671	1.33	.206	1.28	.189	1.29	.173	1.30	.190
	4813	1.35	.184	1.33	.203	1.36	.214	1.35	.201
	4901	1.29	.176	1.31	.197	1.29	.187	1.30	.187
	4914	1.30	.190	1.35	.192	1.33	.171	1.33	.186
	5151	1.28	.163	1.28	.151	1.29	.187	1.28	.168
	5304	1.29	.195	1.31	.204	1.30	.168	1.30	.189
	5412	1.30	.201	1.24	.142	1.31	.195	1.28	.181
	Average	1.30	.186	1.30	.183	1.31	.184	1.30	.184

[1] All average standard deviations in this table were obtained as an average within fleeces by averaging the squared standard deviations and extracting the square root.

TABLE 3.—*Values of χ^2 found from homogeneity tests of variance pertaining to diameter and contour measurements of fiber at different locations on fleece*[1]

Location on fleece	Degrees of freedom	χ^2 for— Diameter: Flesh	Diameter: Midpoint	Diameter: Tip	Contour: Flesh	Contour: Midpoint	Contour: Tip
Shoulder	8	97.53	57.55	96.78	21.16	28.74	20.22
Middle	8	70.42	40.39	160.11	78.36	51.26	44.69
Thigh	8	64.74	48.24	152.22	40.60	41.78	29.91

[1] All χ^2 values in this table exceed 1-percent point.

Table 3 shows the values of χ^2 for a homogeneity test of errors for the diameter and contour measurements when a composite is made of all the fleeces at any one fleece location and position. In the case of the diameter measurements all the composites were heterogeneous. The flesh and midpoint samples were more heterogeneous at the shoulder, and the tip at the middle and thigh locations. The most homogeneous sample was the middle midpoint and the most heterogeneous the middle tip.

In the case of the contour measurements (table 3), all the composites were heterogeneous. In most of the contour measurements the samples did not exhibit as great a tendency to heterogeneity as the diameter measurements.

If the fiber data are grouped according to location on a fleece and χ^2 is computed for the relationship between fiber positions, homogeneity will be found to exist in approximately three-fourths of the diameter measurements (table 4). Thus in all but two fleeces at the shoulder location, two at the middle, and three at the thigh, homogeneity existed between the flesh, midpoint, and tip portions of the fiber of the same fleece

TABLE 4.—*Values of χ^2 found from homogeneity tests of variance pertaining to diameter and contour measurements of fiber from different fleeces*[1]

Fleece No.	Degrees of freedom	χ^2 for Diameter			χ^2 for Contour		
		Shoulder	Middle	Thigh	Shoulder	Middle	Thigh
4190	2	2.88	*7.84	3.28	**24.52	3.29	**9.52
4630	2	1.25	.93	1.51	5.92	3.34	*6.89
4671	2	4.79	.28	**13.01	1.68	2.24	*6.13
4813	2	*7.43	2.97	*7.70	4.14	3.90	4.49
4901	2	.02	.19	.48	**11.42	*7.14	2.55
4914	2	.31	.32	5.93	5.45	.73	2.18
5151	2	3.08	*6.99	1.76	1.30	**23.00	*8.21
5304	2	*8.56	3.31	3.92	4.38	**11.82	*7.42
5412	2	5.98	1.35	**52.13	**25.38	2.88	**22.71

*χ^2 exceeds 5 percent point. **χ^2 exceeds 1 percent point.

As shown in table 4, diameter was more homogeneous throughout the fiber length than contour ratio. Fiber that was homogeneous in diameter measurements was not necessarily homogeneous in contour.

Homogeneity of errors is assumed in the calculations of the analysis of variance, but, as was shown in tables 3 and 4, the individual fleeces and composites did not exhibit this characteristic in all instances. Since, however, the nine fleeces studied were the only ones for which data were available, the analysis of variance was applied, recognizing that the variability of the individual fiber measurements of the different sheep was not of a homogeneous nature.

In table 5 are shown the mean squares for sheep, positions in the fiber, and the interaction of sheep×position (error) of the diameter and contour measurements. Generalizations regarding average diameters and contours for the three positions in the fiber over the entire fleece and for all sheep of the breed may be made from the data in this table. In the case of the diameter measurements, differences between sheep measurements at a single location were highly significant, and differences due to position in the fiber were significant at the middle location only. The mean square value for variation due to error was lowest at the middle location and highest at the thigh.

In the contour ratios reported in table 5, a significant difference between means of fiber measurements at the middle location was found, while all other differences were nonsignificant. The error variance for the thigh location was approximately twice that of the shoulder and middle.

TABLE 5.—*Mean squares for sheep, position in the fiber, and error from analyses of variance of diameter and contour measurements made at three locations on the fleece*[1]

Location	Mean square values for sheep,[2] position,[3] and error[4] at 3 locations					
	Diameter			Contour		
	Sheep	Position	Error	Sheep	Position	Error
Shoulder	**3,726.91	315.11	204.78	0.1351	0.2423	0.0677
Middle	**2,412.19	*614.23	161.83	*.2239	.1819	.0668
Thigh	**4,542.70	1,382.20	575.22	.2696	.0530	.1280

[1] *Exceeds 5 percent point in the *F* test. **Exceeds 1 percent point in the *F* test.
[2] 8° of freedom.
[3] 2° of freedom.
[4] 16° of freedom.

TABLE 6.—*Mean squares for position in fiber and error at three locations from analyses of variance of fiber diameter and contour measurements made for the Notail breed*[1]

Location	Position in fiber	Mean squares for sheep[2] and error[3] in 3 positions at 3 locations			
		Diameter		Contour	
		Sheep	Error	Sheep	Error
Shoulder	Flesh	**1,262.75	23.53	**0.0900	0.0182
	Midpoint	**1,302.75	24.11	**.1605	[illegible]
	Tip	**1,550.75	21.12	.0389	.0229
Middle	Flesh	**800.50	28.40	**.1189	.0224
	Midpoint	**917.2	26.12	**.1944	.0238
	Tip	**1,024.38	24.53	.0486	.0260
Thigh	Flesh	**1,366.12	166.63	**.1626	.0344
	Midpoint	**2,058.88	59.22	**.3864	.0327
	Tip	**2,263.25	58.72	**.1406	.0337

[1] **Exceeds 1 percent point in the *F* test.
[2] 8° of freedom.
[3] 179° of freedom.

Table 6 shows the application of the analysis of variance to the breed composite of the three positions in the fiber at the three locations on the sheep. Separation of the data by position in the fiber for the three locations in the composited fleeces is of importance in indicating whether choice of the position of measurement of diameter and contour is necessary or of significance in fleece grading. Variation due to sheep was found to be highly significant in all cases of diameter measurements, and in all but two cases of contour measurements. The mean-square values due to error were relatively constant for the three positions at the shoulder and middle locations for the diameter measurements, while the value for the flesh thigh was almost three times that of the midpoint or tip thigh.

When the analysis of variance was applied to the diameter studies of variation due to position in the fiber within a single location on a fleece, differences were found to be significant at the 1-percent point in 15 instances, significant at the 5-percent point in 4 instances, and nonsignificant in 8 instances. These results are shown in table 7. In the majority of fleeces studied the mean-square values for variation due to error were appreciably higher at the thigh location than at either the shoulder or the middle locations. The range in significance found for the specific positions within locations on a fleece indicates that the use to which the data would be put will be the deciding factor in sampling fleeces for analysis.

TABLE 7. *Mean squares for position in the fiber and error from analyses of variance of fiber diameter measurements made at 3 locations on the fleece* [1]

Fleece No.	Mean squares for position in fiber [2] and error [3] at 3 locations					
	Shoulder		Middle		Thigh	
		Error	Position	Error	Position	Error
4190	*121.5	32.86	*126.0	35.03	**312.0	38.20
4630	**469.5	23.71	**633.0	32.17	**3,575.0	114.08
4671	23.0	33.23	13.5	16.82	146.5	79.86
4813	**171.0	13.13	**150.0	32.39	**198.5	39.43
4901	**101.0	17.88	**105.0	26.59	*269.5	68.20
4914	9.5	22.13	21.0	26.71	*161.5	50.84
5151	**289.5	17.81	**290.5	25.56	**353.5	49.81
5304	**125.0	25.02	**167.5	19.78	59.0	67.61
5412	15.0	20.50	58.5	22.22	**833.0	66.73

[1] *Exceeds 5 percent point in the *F* test, **exceeds 1 percent in the *F* test.
[2] 2 of freedom.
[3] 597 of freedom.

TABLE 8. *Mean squares for position in the fiber and error from analyses of variance of fiber contour measurements made at 3 locations on the fleece* [1]

Fleece No.	Mean squares for position in fiber [2] and error [3] at 3 locations					
	Shoulder		Middle		Thigh	
	Position	Error	Position	Error	Position	Error
4190	**0.1763	0.0266	0.1030	0.0989	0.0219	0.0291
4630	**.1172	.0144	.0152	.0204	**.3021	.0328
4671	.0269	.0218	.0566	.0315	*.1572	.0363
4813	.0300	.0204	.0321	.0234	.0644	.0401
4901	.0322	.0262	**.1793	.0240	.0200	.0342
4914	**.1546	.0208	.0262	.0231	*.1584	.0343
5151	.0220	.0230	**.1752	.0304	.0178	.0285
5304	**.1907	.0185	**.1271	.0187	.0306	.0357
5412	*.0709	.0198	.0356	.0221	**.2957	.0329

[1] *Exceeds 5 percent point in the *F* test; **exceeds 1 percent point in the *F* test.
[2] 2° of freedom.
[3] 597° of freedom.

In table 8 an analysis of variance is applied to the contour measurements at the three positions in the fiber at a single location on individual sheep. Variation due to position within a single location for contour on a fleece was highly significant in nine instances and significant in three instances. In only three of the fleeces were significant differences found at the middle location on a fleece. With two exceptions variation due to error was higher at the thigh than at either the shoulder or middle location on individual sheep.

Further justification for the application of the analysis of variance where heterogeneity has been shown may be seen by examination of the data in the tables presented above. Using measurements at the shoulder location as an example, while the variances between fibers within individual sheep were heterogenous, the variances for individual sheep ranged from 13.13 to 33.23 (table 7), all numerically small as compared with the variance due to sheep×position (204.78). There can be little question that the variance between sheep is highly significant. The range for individual sheep (13.13 to 33.23) while significant because of the large number of measurements involved, is relatively unimportant because of its small influence in changing the value of the interaction.

SUMMARY AND CONCLUSIONS

Since sampling of wool fleeces, preparation of microscopic slides, fiber measurements, and the necessary calculations are all time-consuming procedures, the present study was undertaken to determine whether in wool-breeding programs the measurement of fiber diameter and contour at more than one point along the fiber length is of importance. The samples of wool were taken from different locations on fleeces of sheep of the Notail breed, exposed to the same environmental conditions and given the same feedstuffs.

Examination of the analysis of variance indicates, in general, that significant differences in diameter of fiber existed between the various sheep of the same breed and that in some fleeces significant differences in the measurements of diameter and contour at various points along the fiber length were present.

Homogeneity of fiber diameter and contour measurements did not exist between fleeces within the breed studied and within positions in the fiber at three locations on the same sheep. Homogeneity was found in approximately three-fourths of the instances when the χ^2 test was applied to studies of wool from the three fiber positions at a single location upon individual sheep, and in more than one-half of the contour measurements of the same grouping.

Applications of the analysis of variance to fleeces and fleece components indicated significant differences involving diameter measurements because of variation due to location on sheep (shoulder, middle, or thigh); variation due to position in the fiber at the middle location; variation due to sheep at the three fiber positions for each fleece location in all instances; and variation due to position in the fiber for approximately two-thirds of the individual fleeces at each location In the contour studies significant differences were found because of variation due to sheep at the middle location; variation due to sheep in all but two instances of positions at each location; and variation due to position in less than half of the fleeces at each location.

From a practical standpoint, the measurement of fiber diameter and contour at all three positions used in the present study would not seem to be necessary in a breeding program where such measurements are used in the preliminary elimination of undesirable or variable animals from the flock. Examination of data obtained by measurements at a single position upon fibers at three locations on the fleeces would indicate whether appreciable variation among locations existed. These same data could be used in comparisons of sheep within a flock.

From the results here reported it may be concluded that in studies of the effects of environment and feed, consideration of variation throughout the length of the wool fiber is of importance. Spinnability of wool is effected by contour and diameter and their variation among and within fibers. Thus, in conducting a wool-breeding program to produce a maximum of desirable fleece qualities, this study indicates that diameter and possibly contour should be measured at more than one point.

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ROOT ROT OF PEPPER AND PUMPKIN CAUSED BY PHYTOPHTHORA CAPSICI[1]

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INTRODUCTION

A destructive root rot of bell or sweet green pepper (*Capsicum annuum* L. var. *grossum* Sendt.) and pumpkin (*Cucurbita pepo* L. var. *condensa* Bailey) plants was observed in a truck section of the San Joaquin Valley, near Merced, Calif., on May 19, 1934 (*7*).[2] In addition to a high mortality, many plants showed sudden and severe wilting, and as the season progressed, there was a marked reduction in stand. The disease was caused by *Phytophthora capsici* Leonian (*5*). Because of the economic importance of these crops, an investigation of the disease was undertaken. The results are presented herewith.

REVIEW OF LITERATURE

In 1922, Leonian (*5*) described a stem and fruit blight of chili pepper, caused by *Phytophthora capsici* sp. nov., which was prevalent in New Mexico. Symptoms of the disease consisted mainly of dry lesions on the pods, blighting of young and older branches, and girdling. While the pathogen did not cause a root rot under field or greenhouse conditions, it sometimes affected some of the younger roots. Introduction of the fungus into the soil failed to cause wilting of healthy pepper plants.

Subsequently, the blight phase of the disease was reported from other States and foreign countries. There are, however, several reports dealing with the root rot phase which are herewith reviewed because of their direct bearing on this paper.

Wilt and desiccation of chili and eggplant seedlings, accompanied by a collar constriction, was recorded in the province of Naples, Italy, in 1924 by Trotter (*11*). The flowers and foliage were not directly affected. The causal organism was identified as *Phytophthora omnivora.* This appears to be the first record of the occurrence of a phytophthora disease of pepper characterized by wilting due to root infection.

In 1927, Curzi, (*1*) described a root rot disease of chilies caused by *Phytophthora hydrophila.* There was evidence that the disease

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was spread by water. Tucker (*12*) has since shown that this species is identical with *P. capsici.*

Sarejanni (*9*) found a serious collar rot of chilies in Macedonia, Greece, in 1933, which was caused by *Phytophthora capsici.* At least 70 percent of the crop was affected. Diseased plants quickly wilted, while occasional attacks were noted on the fruits and on young plants in seedbeds. Infection was favored by heavy watering and the prevailing high summer temperatures.

In Florida, in 1932, Weber (*13*) observed both a blighting of the aerial parts (stems, branches, fruit, and leaves) and a girdling of pepper plants at the soil level by *Phytophthora capsici,* resulting in sudden wilting and death. The latter symptom may have been caused by root infection, although it is not so stated.

According to Kreutzer (*3*), a species of *Phytophthora* (probably *P. capsici*) isolated from rotting cucumber fruits caused severe damping-off of cucumber and pepper seedlings. Also, 10 to 20 days after the fungus was introduced into soil in which mature pepper plants were growing, the plants developed a severe blight.

In a recent annual report of the Colorado Agricultural Experiment Station (*8*), is was stated that *Phytopthora capsici,* obtained from a fruit rot of tomato, had been shown to cause a wilt of pepper plants. In Weld county, the same organism was responsible for a wilt of squash and watermelon.

The results of further studies on a decay of cucumber and tomato fruits and on a wilt of squash and watermelon vines, caused by *Phytophthora capsici,* were recently published by Kreutzer, Bodine, and Durrell (*4*).

Tompkins and Tucker (*10*) showed that an isolate of *Phytophthora capsici* from a honeydew melon caused wilting of young pepper plants in the greenhouse within 10 days after its introduction into the soil, and after 20 days all were badly wilted and dying.

Godoy (*2*) in a recent paper mentioned the occurrence of damping-off of pepper seedlings in Argentina.

FACTORS FAVORING INFECTION

Pepper and pumpkin plants require abundant soil moisture, good drainage, and relatively high air temperatures for optimum growth. By the same token, these are the principal environmental factors which favor inception and spread of the root rot disease in the truck sections near Merced. In most cases, these crops are grown along river bottoms and in low areas which, although rich in fertility, lack adequate drainage. With frequent, heavy, surface irrigation, necessitated by the growth requirements of the plants, the soil quickly becomes excessively wet and often waterlogged.

It has been observed that when plants are grown on higher, well-drained ground and supplied with only enough moisture to satisfy growth requirements, then the incidence of disease is greatly reduced.

SYMPTOMS OF THE DISEASE

Field observations and greenhouse tests under controlled conditions indicated that infection of pepper and pumpkin plans by *Phytophthora capsici* results in symptoms common to both. Although a few plants die early in the season when temperatures are naturally lower, a high incidence of disease is uncommon until the advent of high temperatures at midseason, at which time many plants have reached the fruiting stage. Infection continues on a high level during the rest of season. The disease appears very suddenly, and infected plants usually die promptly.

Above ground, the chief diagnostic symptoms of the disease consist of a rapid, permanent wilting of the leaves, without noticeable change in color; a blackish-brown discoloration of the lower part of the stem, adjacent to the soil level; and collapse of the stem, followed by lodging of the plant on the ground.

Under ground, the invaded roots and stems show a soft, water-soaked, blackish-brown, odorless type of decay which ultimately involves all tissues. Diseased plants are easily pulled from the soil, but invariably the cortex of the taproot and lateral roots sloughs off and remains behind.

THE CAUSAL FUNGUS, PHYTOPHTHORA CAPSICI

ISOLATION

The causal fungus, *Phytophthora capsici*, was readily isolated from the roots and basal part of the stems of recently infected pepper and pumpkin plants by removing aseptically small tissue fragments from the internal, advancing margin of decay, planting on either prune or malt-extract agar (*6*), and incubating at room temperature. Also, small sections of fine lateral roots, after thorough washing, were planted directly on agar. After 48 hours, pure cultures of the fungus were established on agar slants by transferring hyphal tips from the edge of the Petri-dish colonies. The fungus was cultured at intervals from several lots of naturally infected specimens, involving about 100 pepper and 50 pumpkin plants. Pure cultures of *P. capsici* were constantly obtained from tissue plantings, and there was no indication of the presence of other species.

IDENTITY AND TEMPERATURE RELATIONS

Comparative studies were made on three isolates from Yellow Crookneck pumpkin, one from Summer pumpkin, and two from bell pepper (var. Barbanera no. 1). All isolates were secured from the roots and lower portions of stems of diseased plants in the Merced area. The isolates exhibited the usual slight variations in type and rapidity of growth in agar plates, in tendency to develop sporangia and oogonia, and in size of reproductive organs. The isolates from pumpkin could not be distinguished from those from pepper.

The sporangia were papillate, limoniform to irregular or elongate, with a mean size of 41 by 30 microns when produced in oatmeal-agar cultures. In liquid cultures the sporangia were somewhat larger and produced zoospores more frequently than when developed on agar media. Chlamydospores were not observed, but in oogonium-producing cultures occasional unfertilized oogonia germinated by germ tubes in the manner characteristic of chlamydospores.

Oogonia and oospores were formed in large numbers by one isolate, while two failed to develop them even after 3 months' incubation at 15° C. The remaining three isolates developed a few oogonia and oospores in old cultures. The antheridia were of the permanent, amphigynous type, encircling the stalk of the oogonium.

The temperature-growth relations of the isolates were observed after 96 hours' growth on Difco corn-meal agar plates (pH 6.1). All grew well at temperatures ranging from 15° to 35° C. The optimum temperature appeared to lie between 25° and 30°, two isolates showing slightly greater growth at the former and four at the latter temperature. Minor differences were observed in growth rates, and in the appearance of the mycelial weft, which varied from regularly radiate growth to a matted, slightly irregular type. Some isolates exhibited both types of growth when incubated at different temperatures.

The isolates from pumpkin and pepper corresponded very well in all characters with isolates of *Phytophthora capsici* from pepper studied under comparable conditions by Tucker (*12*), with isolates from honeydew melons reported by Tompkins and Tucker (*10*), and with isolates from Winter Queen watermelons studied by Wiant and Tucker (*14*).

PATHOGENICITY TESTS

Pure cultures of *Phytophthora capsici*, isolated from the roots of naturally infected Barbanera No. 1 pepper and Early White Bush Scallop, Zucchini, and Yellow Crookneck pumpkin plants, were used in the infection experiments. Inoculum was prepared by growing the pepper and pumpkin isolates of the fungus on sterilized, moistened cracked wheat. It was added in uniform quantity to autoclaved soil in 6-inch pots, each containing a young plant, in a manner designed to avoid wounding the roots. Sterilized wheat was used for the noninoculated controls. The plants, grown in a greenhouse at temperatures ranging from 28° to 32° C., were watered heavily each day to provide favorable conditions for infection.

ON PEPPER

The pathogenicity of three pepper isolates was proved on the variety Barbanera No. 1, because this pepper is grown extensively in the Merced section. On July 18, 1934, 25 pepper plants were inoculated with each isolate. By August 1, all plants were infected, the incubation period ranging from 6 to 14 days. Symptoms shown by diseased plants were identical with those resulting from natural infection (fig. 1, *A*). Typical wilting of the leaves, destruction of the root system, and browning of the basal part of the stem with lodging of the plant occurred. When diseased plants were pulled from the soil, the soft, watery, blackish-brown cortical tissues sloughed off from the central cylinder of the taproot (fig. 2, *A*). Most of the lateral roots were completely rotted, in marked contrast to the healthy condition (fig. 2, *B*). The 15 noninoculated controls continued healthy (fig. 1, *B*). All infected roots yielded the fungus when plated.

On August 17, a total of 60 pepper plants of the same variety were inoculated with the reisolates. All plants were infected by August 27, the incubation period ranging from 5 to 10 days. The 10 noninoculated controls remained healthy.

Damping-off of Barbanera No. 1 pepper seedlings occurred when seeds were planted in previously infested soil or in autoclaved soil to which the fungus isolates were later added.

ON PUMPKIN

The pathogenicity of the pumpkin isolates was proved on the varieties Early White Bush Scallop, Zucchini, and Yellow Crookneck, the seeds of which had been obtained from a grower in the Merced section. On August 10, 1934, 25 pumpkin plants of each variety were inoculated with each isolate. By August 31, all plants were infected, the incubation period ranging from 12 to 21 days. The symptoms shown by diseased plants were identical with those resulting from natural infection and followed quite closely the sequence described for artificially infected pepper plants. The fungus was reisolated from all infected plants. The five noninoculated controls of each variety remained healthy.

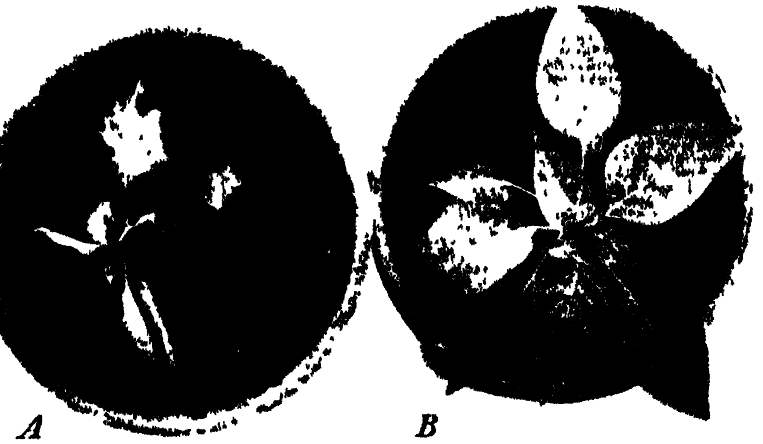

FIGURE 1.—Phytophthora root rot of pepper and pumpkin: *A*, Artificial infection of the pepper variety Barbanera No. 1 after 8 days, showing rapid wilting and collapse of the plant; *B*, healthy control.

On September 15, 20 pumpkin plants of each of the four varieties were inoculated with the reisolates, and all plants became diseased by September 25. The incubation period ranged from 5 to 10 days. The noninoculated controls remained healthy.

Similarly, damping-off of Early White Bush Scallop, Zucchini, and Yellow Crookneck pumpkin seedlings occurred, as mentioned above for pepper.

CROSS INOCULATIONS

In parallel inoculations, three isolates of *Phytophthora capsici* from pepper proved pathogenic to Early White Bush Scallop, Zucchini, and Yellow Crookneck plants in the greenhouse, and, likewise, two isolates each from Early White Bush Scallop, Zucchini, and

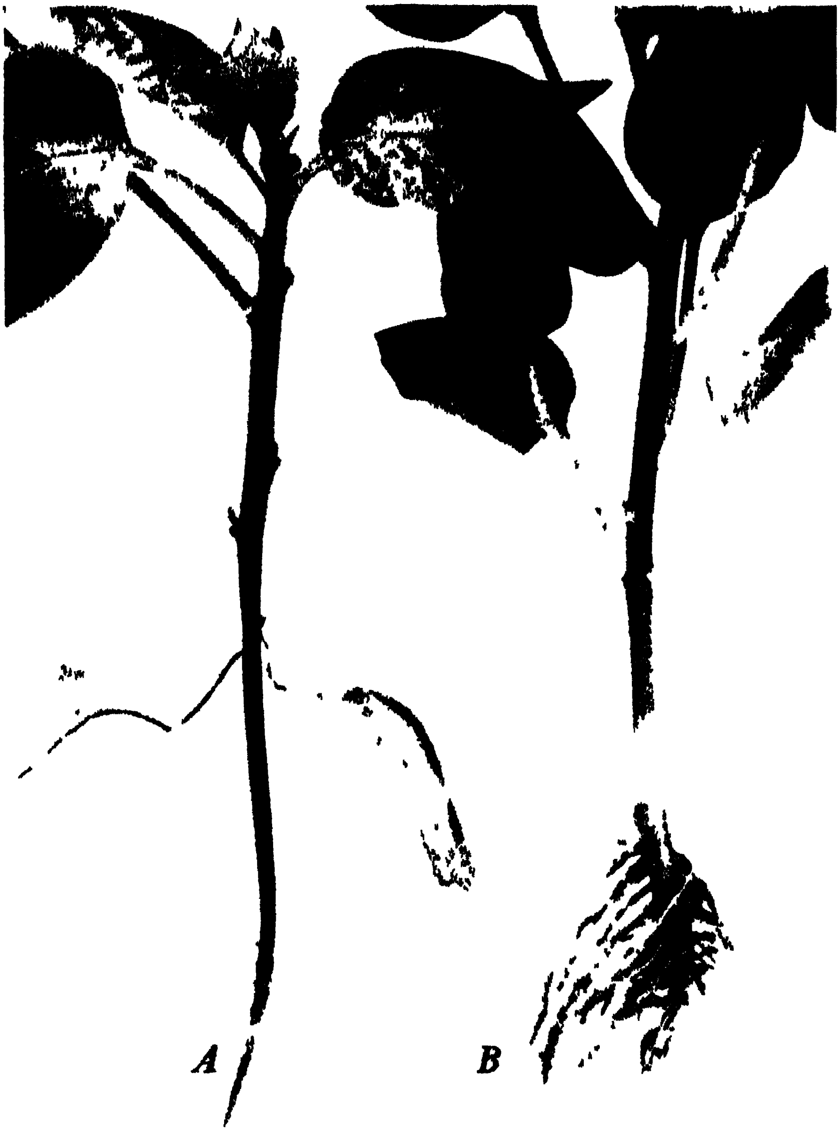

FIGURE 2.—Phytophthora root rot of pepper and pumpkin. *A*, Blackening of the stem and decay of the root system of young Barbaneta No. 1 pepper plant; *B*, healthy control.

Yellow Crookneck pumpkin caused infection of pepper plants (fig. 3), with incubation periods closely corresponding to those just mentioned. It may be assumed, therefore, that these isolates, based upon their infective capacity, are very similar, if not identical.

These isolates were also tested by inoculating healthy Zucchini pumpkin and pepper fruits in the laboratory, using the technique described elsewhere (*10*). All isolates induced a severe type of fruit rot.

SUSCEPTIBILITY OF PEPPER AND PUMPKIN VARIETIES TO INFECTION

Numerous commercial varieties of pepper and pumpkin were tested for susceptibility to infection with their respective isolates of *Phytophthora capsici.* No fewer than 20 plants of each variety were inoculated according to the procedure previously described, involving in the aggregate about 2,500 pepper and 300 pumpkin plants. All varieties proved to be highly susceptible, with no survival of individuals in any particular variety. The incubation period for pepper varieties ranged from about 5 to 49 days; for pumpkin varieties, from 4 to 33 days. When considered collectively, the minimum incubation period for pepper varieties was 8 days and the maximum 29 days; for pumpkin plants, the minimum was 8 days and the maximum 23 days

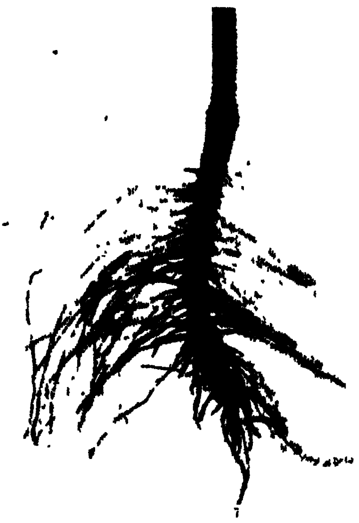

FIGURE 3. Phytophthora root rot of pepper and pumpkin. Artificial infection of young pepper plant, after 16 days, with an isolate of *Phytophthora capsici* from Zucchini pumpkin, showing a blackish brown discoloration of the basal part of the taproot and some lateral roots.

In the winter of 1937, seeds which had been collected at Merced from apparently resistant Barbanera No. 1 pepper plants were planted in autoclaved soil in the greenhouse. The progeny proved highly susceptible upon inoculation.

The following varieties of pepper were tested in the greenhouse: Anaheim, Asgrow King, Bell or Bull Nose Hot, Bell or Bull Nose Sweet, Birdseye or Coral Gem, Black Long of Mexico, Bountiful No. 7, Bulgarian, California Wonder, Cayenne, Cayenne Large Thick, Cayenne Long Narrow, Celestial, Cherry, Chinese Giant, Colossal, Crimson Giant, Dwarf Chili Red Cluster, Early Giant, Early Mountain, Elephant Trunk, Floral Gem, Giant of China Golden Yellow, Giant of China Red, Gloria, Golden Dawn, Golden Queen, Green

Mexican Pickling, Green Pointed Sharp, Harris Earliest, Harris Early Giant, Heiffer Horn, Hercules, Hungarian Hot or Banana, Hungarian Yellow Wax, Kaleidoscope, Kalinko, King of the North, Large Bell or Bull Nose, Large Early Neapolitan, Large Long Red Cayenne, Large Mexican Chili, Long Hot or Finger, Long Podded, Long Red Cayenne, Mexican Chili, Mile High Sweet Giant, Monstrous, Narrow Podded Neapolitan, Neapolitan Giant, New Victory, Nikko Togarashi, Nocera Golden Yellow, Nocera Large Red, Nordico, Ohio Crimson Giant, Oshkosh, Pimiento, Prince of Wales, Prizetaker, Procopp's Giant Red, Proteus, Red Cherry, Red Chili, Red Cluster, Red Hercules, Rosse, Royal King, Ruby Giant, Ruby King, Ruby King Special, Spanish Bull, Spanish Gem, Square Yellow Big of Naples, Squash or Tomato Hot, Squash or Tomato Shaped, Squash or Tomato Sweet, Sudanese Chili, Sunnybrook, Sunnybrook Sweet Cheese, Sweet Meat Glory, Sweet Mountain or Mammoth, Sweet Salad Tomato, Sweet Spanish, Tabasco, Taka-No-Tsume, Tomato or Squash, Tomato Shaped Red Squash, Tom Thumb Round Scarlet, Verde, Verticus, White Stump Sweet, Windsor A, B, C, D, E, and G (selections from the Connecticut Agricultural Experiment Station), World Beater, Yatsu-Fusa, Yellow Hercules, and Yellow Oshkosh.

The following varieties of pumpkin were tested in the greenhouse: Early Green Bush Scallop, Early White Bush Scallop, Early Yellow Bush Scallop, Giant Summer Crookneck, Italian Marrow Cocozelle, Italian Marrow Zucchini, Mammoth Summer Crookneck, Table Queen, and Yellow Summer Crookneck.

EXPERIMENTAL HOST RANGE

Young plants of squash (*Cucurbita maxima* Duchesne), eggplant (*Solanum melongena* L. var. *esculentum* Nees), tomato (*Lycopersicum esculentum* Mill. var. *vulgare* Bailey), and Turkish tobacco (*Nicotiana tabacum* L.) were tested in the greenhouse for susceptibility to infection with the pepper and pumpkin isolates of *Phytophthora capsici*, using the inoculation technique previously described. A suitable number of noninoculated controls was reserved for each variety tested, and during the experiments they continued healthy. Reisolations were made from all diseased specimens, and the reisolated fungus from a particular host was then tested by inoculation into healthy specimens of that host.

The following varieties of squash proved highly susceptible: Banana, Blue Hubbard, Boston Marrow, Delicious, Delicious Golden, Golden Hubbard, Hubbard, and Warted Hubbard.

Of the five varieties of eggplant used, only two were shown to be susceptible, i. e., Black Beauty and Large New York Purple. No infection was obtained on Colossal, Florida High Bush, and Improved Large Purple.

Only one variety of tomato (Early Santa Clara Canner) was tested, and it proved highly susceptible.

None of the isolates caused infection of Turkish tobacco plants.

Tubers of potato (*Solanum tuberosum* L.) var. Russet Burbank were inoculated with pure cultures of the five isolates in the laboratory, but they remained healthy.

SUMMARY

A root rot of pepper and pumpkin plants, caused by *Phytophthora capsici* Leonian, is prevalent in the San Joaquin Valley, near Merced, Calif.

Excessive moisture, inadequate soil drainage, and high air temperatures definitely favor the inception and spread of the disease.

The root system and the basal part of the stems of pepper and pumpkin plants show a soft, wet decay. Permanent wilting of the leaves occurs very suddenly, with ultimate collapse of the stem and lodging and death of the plants.

The isolates of the fungus from pepper and pumpkin are described.

Infection was obtained in the greenhouse by adding the fungus to the wet, autoclaved soil of potted plants. The incubation period ranged from 6 to 14 days for pepper and from 12 to 21 days for pumpkin plants.

Successful cross inoculations were made with pepper isolates to pumpkin, and vice versa. All isolates caused damping-off of pepper and pumpkin seedlings in the greenhouse and rotting of pepper and pumpkin fruits in the laboratory.

The pepper and pumpkin isolates of *Phytophthora capsici* were also pathogenic to squash, eggplant, and tomato plants.

No resistance to the disease was found in any of the varieties of pepper and pumpkin tested under greenhouse conditions.

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THE CHEMICAL COMPOSITION AND APPARENT DIGESTIBILITY OF NUTRIENTS IN SMOOTH BROMEGRASS HARVESTED IN THREE STAGES OF MATURITY[1]

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INTRODUCTION

Smooth bromegrass (*Bromus inermis* Leyss.), a very leafy, sod-forming, long-lived perennial, ranks high among the cultivated grasses in palatability and yielding qualities. It requires more moisture for growth than crested wheatgrass (*Agropyron cristatum* (L.) Beauv.) and should not be considered a competitor of this grass in the drier areas. Because of its importance in pure stands and in mixtures with alfalfa and other legumes and grasses, both as a pasture and a hay crop, it seemed advisable to undertake a study of the chemical life history and the digestibility of the nutrients at various stages of maturity of this grass when grown under the soil and climatic conditions of the Palouse area of eastern Washington.

REVIEW OF LITERATURE

Smooth bromegrass has persisted for 20 years in a horse paddock at the Illinois Agricultural Experiment Station (*1*).[2] At the same station (*2*, *5*) 470-pound calves consumed 30 pounds of green grass containing 28.3 percent of dry matter and 850-pound steers consumed 52.7 pounds of the same grass daily. Smooth bromegrass in these studies contained 1,023 calories of digestible energy per pound of dry matter, and with a 68.29-percent availability, the net energy value was 699 calories. Field records at the Illinois Station (*1*) show smooth bromegrass to excel timothy, orchard grass, bluegrass, and reed canary grass in carrying capacity for yearling Hereford heifers. The gains produced during the first month of grazing were greater than those made during the remainder of the grazing season.

Under western dry-land conditions smooth bromegrass pasture does not produce the gains obtained from crested wheatgrass pasture. Orcutt (*7*) at the Moccasin substation in Montana recorded gains of 61 pounds per acre on crested wheatgrass pasture and 41.5 pounds on smooth bromegrass pasture when steers were grazed in 23.6-acre plots at the rate of four to eight steers per plot. When the value of old native grass pasture was rated as 100 percent, seedings of smooth bromegrass and crested wheatgrass had values of 112 and 177 percent, respectively.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 431.

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Shepperd (*9*) at the North Dakota Experiment Station reported daily gains of 1.86 pounds per head when steers were grazed on second-year sweetclover as compared with gains of 1.54 pounds on smooth bromegrass pasture.

Studies by Hopper and Nesbitt (*3*) of the more mature stages of smooth bromegrass showed a protein content of 8.80 percent at blossoming time and a crude fiber content of 29.96 percent. Before heading it contained 15.14 percent protein and 23.56 percent crude fiber. Ladd (*4*) reported that smooth bromegrass hay contained 6.56 percent ash, 6.22 percent fat, 28.11 percent crude fiber, 13.60 percent protein, and 45.51 percent nitrogen-free extract. This hay was twice as rich in crude protein as timothy hay.

Nelson and Shepherd (*6*) observed that seeding a legume with smooth bromegrass prevents a "sod-bound" condition, and Rather and coworkers (*8*) observed that smooth bromegrass in mixtures with legumes speeds up the rate of curing of hay, and reduces danger of bloat when the mixtures are pastured.

Waldron and Porter (*12*) noted that at the North Dakota Station smooth bromegrass stays succulent longer than other grasses with the advent of warm weather.

PLAN OF THE INVESTIGATION

Samples of the grass were collected at approximately 2-week intervals and changes in chemical composition studied throughout the entire growing season. Samples of grass cut at a height of 4, 10, and 36 inches were fed to sheep in digestion stalls for the purpose of studying changes in the digestibility of the nutrients as the grass matured. The 36-inch growth coincided with the anther-falling stage, which ordinarily is that recommended as ideal in hay production.

Ten-day preliminary periods followed by 10-day experimental periods were employed in feeding each sample of forage. The equipment and detailed technique have been fully discussed elsewhere (*10*). The lambs were out of Lincoln X Merino ewes and sired by Hampshire rams.

DEVELOPMENT OF SMOOTH BROMEGRASS PLANTS

The rate of growth at various stages in the development of smooth bromegrass plants is shown in table 1. A moisture content of 72.38 percent at a height of 4.5 inches was reduced to 40.98 percent at the time the seeds were fully mature and the plants were 36 inches tall. The data on the composition of the dry matter in the forage samples is also summarized in table 1. The protein content of the early-spring samples was 13.61 percent, but this declined to 4.02 percent in the forage samples taken at the seed stage. As the protein content decreased the percentage of crude fiber increased from 19.77 to 35.92. The percentage of calcium declined during the season from 0.37 to 0.24 and the percentage of phosphorus declined from 0.32 to 0.13. Table 1 shows that the grass was quite nutritious up to the water stage of seed formation. There was no effective rainfall during the growing season of 1939; it was drier than any similar period in 46 years.

TABLE 1.—*Moisture content and chemical composition of smooth bromegrass clippings as related to height of plants and stage of maturity at approximately 2-week intervals during the growing season of 1939*

Date	Height of plants	Stage of maturity	Moisture content	Chemical composition on dry-weight basis						
				Protein	Crude fiber	N-free extract	Ether extract	Ash	Ca	P
	In		*Pct*	*Pct*	*Pct*	*Pct*	*Pct*	*Pct*	*Pct*	*Pct*
May 3	4.5		72.38	13.61	19.77	53.41	3.73	9.48	0.37	0.32
17	10.0	Vegetative	73.53	13.20	21.76	52.44	3.40	9.14	.29	.32
31	16.5	⅓ headed	61.16	11.31						
June 15	24.0	Heads well formed	71.71	8.16	30.22	51.12	2.37	8.13	.25	.24
30	36.0	10 percent anthers	61.85	7.21	29.65	53.00	1.86	8.28	.30	.20
July 15	36.0	Water stage of seed	52.75	6.86	28.93	55.27	1.38	7.56	.21	.19
30	36.0	Seeds hard	43.93	5.10	29.84	54.40	1.80	8.86	.24	.18
Aug 15	36.0	Seeds fully mature	40.98	4.02	35.92	52.00	1.59	6.47	.24	.13

EXPERIMENTAL RESULTS

The chemical composition of the grass clippings is shown in table 2. A complete summary of the number of lambs used, their weights, their dry matter intake, as well as coefficients of apparent digestibility for each nutrient as determined with individual lambs is presented in table 3.

Only three lambs were fed the 4-inch grass because of the large area required to supply forage at this stage. The dry-matter intake of lambs fed grass at the 4- and 10-inch stages is fairly comparable. At the mature stage the lambs consumed much less dry matter. With roughage rations (*10*) this inequality of dry-matter intake is not a disturbing factor in comparisons of coefficients of digestibility.

The average coefficients of apparent digestibility of the various nutrients are very nearly the same for the samples taken at the 4- and 10-inch stages. In this experiment smooth bromegrass up to the 10-inch stage retained its highly digestible character. A rather marked decrease in digestibility was noted at the hay stage. The grass 4 inches tall had a coefficient of 82 for its crude protein while at a 36-inch height the coefficient was 53. Crude fiber of the 4-inch grass was 85 percent digestible and at the 36-inch stage of growth only 56 percent digestible. A similar decline has been noted for the other nutrients.

TABLE 2.—*Chemical composition of smooth bromegrass at different stages of maturity, fed to lambs during digestion experiments*

Height of grass (inches)	Dry matter	Ash	Protein (N×6.25)	Crude fiber	N-free extract	Ether extract
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
4	37.20	10.67	14.65	20.81	49.35	4.52
10	37.48	7.50	12.06	28.31	48.93	3.20
36[1]	85.45	8.43	6.23	35.55	47.45	2.34

[1] Anther-falling stage

Table 3 also contains figures showing the percent of digestible crude protein content and the total digestible nutrient content of the fresh forage samples studied. By delaying grazing until the

10-inch stage, the nutritive qualities do not undergo much decline, while the carrying capacity of a given area is greatly increased through growth.

TABLE 3.—*Apparent digestibility of the nutrients in smooth bromegrass forage clipped at various stages of maturity and fed to lambs*

Height of grass (inches)	Lamb No.	Live weight of lambs: Initial	Live weight of lambs: Final	Daily dry matter intake	Apparent digestibility: Dry matter	Apparent digestibility: Crude protein	Apparent digestibility: Crude fiber	Apparent digestibility: N-free extract	Apparent digestibility: Ether extract	Total dry matter in forage	Digestible nutrient in fresh forage samples: Crude protein	Digestible nutrient in fresh forage samples: Total	Nutritive ratio 1 to
		Kilograms	*Kilograms*	*Grams*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	
4	1	40.4	42.0	1,116	81	82	84	89	65				
	2	45.4	45.8	1,168	80	82	86	88	64				
	3	43.8	45.4	1,146	82	81	87	89	69				
Average					81.0	81.7	85.0	88.7	66.0	37.20	4.1	20.82	7.70
10	1	41.3	42.2	975	82	80	87	86	63				
	2	45.8	47.8	1,020	81	80	81	86	[illegible]				
	3	39.9	41.1	97	80	78	82	87	60				
	4	44.9	44	933	79	74	76	84	9				
	5	44.7	45.8	1,046	81	80	84	85	62				
	6	47.2	47.4	977	82	81	86	86	63				
Average					80.8	78.8	82.8	87.3	67.0	37.48	3.6	21.71	7.3
[1]	1	40.4	38.6	531	54	71	53	63	20				
	2	4 1	46.7	616	60	5	61	67	31				
	3	47.5	4 1	510	73		72	62	27				
	4	47.6	50.8	687	56	50	5	64	30				
		52.2	52.2	716	54	73	6	64	27				
	6	48.8	49.0	612	56	54	56	63	30				
Average					5 8	53.0	76.0	63.8	27	8.4	.82	46.30	1 12

[1] Anther falling stage.

In table 4 the digestible nutrient content of the dry matter of smooth bromegrass at three stages is contrasted with identical stages of crested wheatgrass as reported in a previous publication (*11*). A study of this table shows that the percentages of digestible nutrients in the dry matter of smooth bromegrass at the 4- and 10-inch stages are as high as in the common concentrates, like corn and barley. Furthermore, smooth bromegrass retains its high nutritive qualities to a more advanced stage of maturity than does crested wheatgrass, and at comparable stages it is a richer source of digestible crude protein and total digestible nutrients. Table 4 shows convincingly the superior nutritive qualities of smooth bromegrass over crested wheatgrass when these two grasses are produced under the soil and climatic conditions of the Palouse area of eastern Washington. However, each is peculiarly adapted to its own environment, smooth bromegrass to the more moist areas and crested wheatgrass to the drier areas. Crested wheatgrass has been highly praised in areas where smooth bromegrass cannot be grown successfully.

TABLE 4.—*Digestible nutrients in the dry matter of smooth brome-grass and of crested wheatgrass harvested at three stages of maturity*

Grass and height when clipped (inches)	Protein (N×6.25)	Crude fiber	N-free extract	Ether extract	Total digestible nutrients
Smooth bromegrass	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
4	11.97	17.69	43.77	2.98	80.14
10	9.50	23.44	41.74	2.14	79.50
36 [1]	3.30	19.91	30.27	.64	54.92
Crested wheatgrass (*11*)					
4	10.15	16.05	36.36	1.85	66.73
10	4.93	19.05	40.09	1.16	66.68
([1])	1.94	19.23	26.06	.34	48.00

[1] Anther falling stage

SUMMARY AND CONCLUSIONS

The protein content of the dry matter of smooth bromegrass clippings, taken at regular intervals throughout the growing season, decreased progressively from 13.61 percent to 4.02 percent. Crude fiber increased from 19.77 percent to 35.92 percent and plant height from 4.5 to 36 inches. The calcium content declined from 0.37 to 0.24 percent and phosphorus from 0.32 to 0.13 percent during the same interval.

By delaying the grazing of smooth bromegrass until it is 10 inches tall, the nutritive qualities do not decline and the carrying capacity appears to be greatly increased. Bromegrass up to a height of 10 inches retains its highly digestible character. The dry matter was 81 percent digestible at the 4- and 10-inch stages and only 55.8 percent digestible at the 36-inch stage. Crude protein, crude fiber, and nitrogen-free extract followed similar trends. Smooth bromegrass retains its high nutritive qualities to a more advanced stage of maturity than does crested wheatgrass and at identical stages of growth is superior to it in digestible crude protein and total digestible nutrients.

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INHERITANCE OF SEED CHARACTERS IN WATERMELON[1]

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INTRODUCTION

Although seed characters have nothing to do with the flavor and texture of flesh of the watermelon (*Citrullus vulgaris* Schrad.) they are of considerable importance in determining its appearance. The belief is widespread that black seeds denote complete maturity and high sugar content; yet many important commercial varieties have white, brown, tan, or speckled seeds. Despite the availability of several different seed sizes, there seems to be a definite preference for large seeds.

Relatively little information is available concerning inheritance in the watermelon; however, intensive breeding operations are under way in many States, and the need for genetic data has become increasingly urgent. Genetic information, in addition to its botanical value, is essential for the most rapid breeding progress. The investigations herein reported are confined to studies on the inheritance of seed length and seed-coat color.

REVIEW OF LITERATURE

No published reference to genetical analysis of seed-length factors has been found. Weetman (*9*)[2] approximated such studies in populations derived from a cross between races wherein weight samples of 25 seeds were light (1.22±0.01 gm.) and heavy (2.60±0.05 gm.). In the F_2 and backcrosses to both parents the lighter weight parent approached monogenic dominance over the heavier, but chi-square analysis failed to establish an acceptable fit between his observed and calculated ratios.

Kanda (*3*) crossed flat black with stippled black and demonstrated as far as F_3 that flat is dominant to stippled by a single-factor difference. A second cross, which also included flat vs. stippled as well as several other characters and for which he postulated four pairs of genes, was carried to an F_2 population of 123 plants. A chi-square analysis of his data shows a significant deviation from this hypothesis, as will be discussed in an appropriate section. McKay (*4*) investigated two crosses, tan × red and green × red, the former phenotype in each cross being dominant to the latter by a single-gene difference. Porter (*8*) demonstrated a single-gene difference of black dominant over tan and tan dominant over white, with the backcross of black × tan to tan giving

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[1] Received for publication February 28, 1941. These studies were initiated in 1930 by D. R. Porter at Davis, Calif. Certain F_1, F_2, and backcross populations were grown there until 1935. Beginning in 1936, the investigations were conducted by the U. S. Department of Agriculture at the U. S. Regional Vegetable Breeding Laboratory, Charleston, S. C.

[2] Italic numbers in parentheses refer to Literature Cited, p. 456.

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1 black to 1 tan. Weetman (*9*) investigated three crosses involving the phenotypes which in our study are called black × clump and tan × clump. In the first case black was dominant to tan by one gene, but in the second case the F_1 phenotype was black and the F_2 segregated into 9 black to 3 tan to 3 clump to 1 white-tan-tip (according to the nomenclature adopted in the present study). In the last case Weetman demonstrated that F_1 × tan gives 1 black to 1 tan, and F_1 × clump gives 1 black to 1 clump.

MATERIALS AND METHODS

Inbred parent stocks used for the crosses were rendered as homozygous as possible before hybridization, but since some inbred lines were selected for specific characters (sugar content, etc.) occasional F_2 populations analyzed for seed characters disclosed a few lines (Sun Moon and Stars and Peerless) that were still heterozygous for seed-color genes. Among the different stocks and varieties of *Citrullus vulgaris*, seed length ranges from 5.5 to 15.5 mm. and seed-coat colors range from an almost pure white through red, green, tan, mahogany, and black, with various patterns superimposed. Seed length and seed-coat colors for varieties used in this study are indicated in table 1. The Sun Moon and Stars variety is represented by one strain in which seed pigment is clumped and another in which it is white-tan-tip-colored; Peerless has one strain with white-tan-tip seeds and another with white-pink-tip seeds. The Striped Klondike variety also showed unstable colors, but the strain used as parent in the studies herein reported showed only clump, a phenotype varying in segregating populations from a large central eyespot to nearly uncolored seeds except for black spots on the hilum prominences.

Figures 1 and 2 are photographs of the seed types segregating in two F_2 populations. Both illustrate the three seed-length phenotypes short, medium, and long—and between them are shown the six color phenotypes used in parents or which segregated from crosses. Figure 1 shows flat black, stippled or dotted black, tan, clump, and white-tan-tip; figure 2 shows tan, red, white-tan-tip, and white-pink-tip.

TABLE 1.—*Varieties of watermelon and phenotypes used in studying inheritance of seed-coat color and seed length*

Seed-coat color	Distribution of varieties having indicated approximate range in seed length		
	Short, 4.5–7.5 mm	Medium, 7.5–11.5 mm	Long, 11.5–16.5 mm
Black		Klondike Winter Queen	Hope Giant
Tan	Baby Delight		Thurmond Grey
Clump		Striped Klondike	Golden Honey Sun Moon and Stars Northern Sweet
White-tan-tip			Chilean Gandy Sun Moon and Stars Peerless Pride of Muscatine
White-pink-tip	--	--	Peerless

Phenotypes of watermelon seed-coat color are sometimes difficult to classify because of variable degrees of expression. The ordinary fluctuation may be accentuated by other factors, such as stage of maturity (especially fermentation) and sunburn either on whole or on broken fruits. A customarily easily classified color, such as red or white-tan-tip, is rendered difficult to classify in populations that rotted before seed collection.

Classification of seed-coat color requires time and patience to match and rematch samples from different fruits, and it is best done in the laboratory from cleaned and dried material.

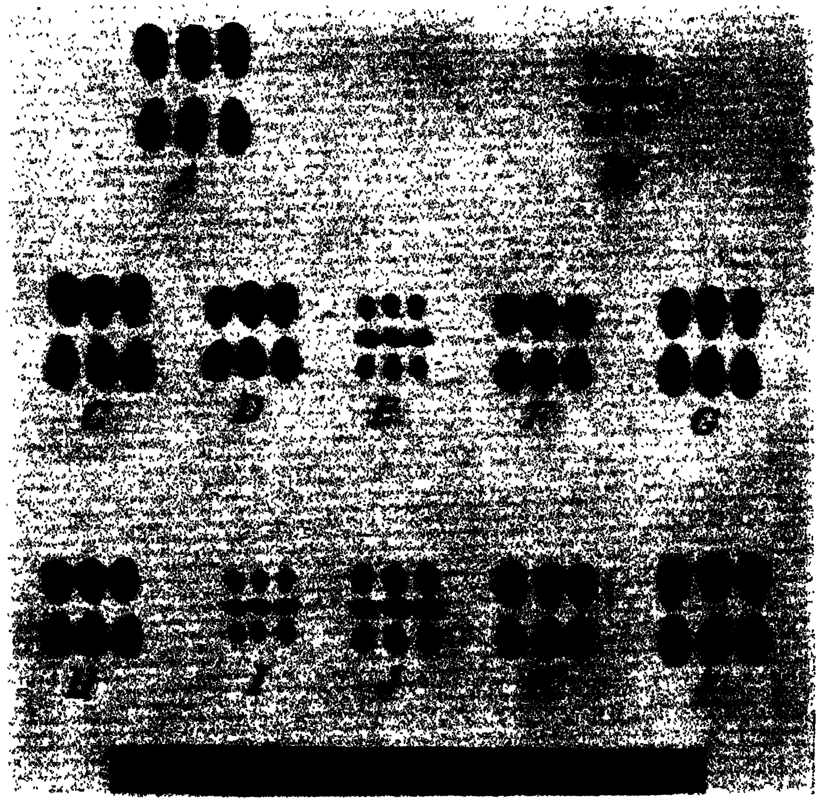

Figure 1.—Seed phenotypes segregating in an F_2 population: *A*, Clump long; *B*, tan short; *C*, flat black long; *D*, flat black medium; *E*, flat black short; *F*, stippled or dotted black medium; *G*, dotted black long; *H*, tan medium; *I*, tan short; *J*, clump short; *K*, clump medium; *L*, white-tan-tip long. For phenotypic ratio, see figure 13.

Seed samples were collected from each fruit and were stored in envelopes with cellophane windows (fig. 3). The measurements of 5 or 10 seeds, depending on the uniformity of the sample, were then recorded on the outside of the envelope. The measurements were made with a vernier caliper to tenths of a millimeter, and the genetic analysis was based on the nature of the frequency distribution obtained. The envelopes of an entire population may be spread on the floor or on a large table, as in figure 4, to present a simultaneous view of the seed-coat color and length and to facilitate the necessary color matching.

All Mendelian ratios were tested for goodness of fit by orthogonalized chi-square formulas developed by Fisher (*1*) and applied extensively by Mather (*6*). This sensitive treatment of the standard method disassembles chi-square values into the component values contributed by each degree of freedom present.

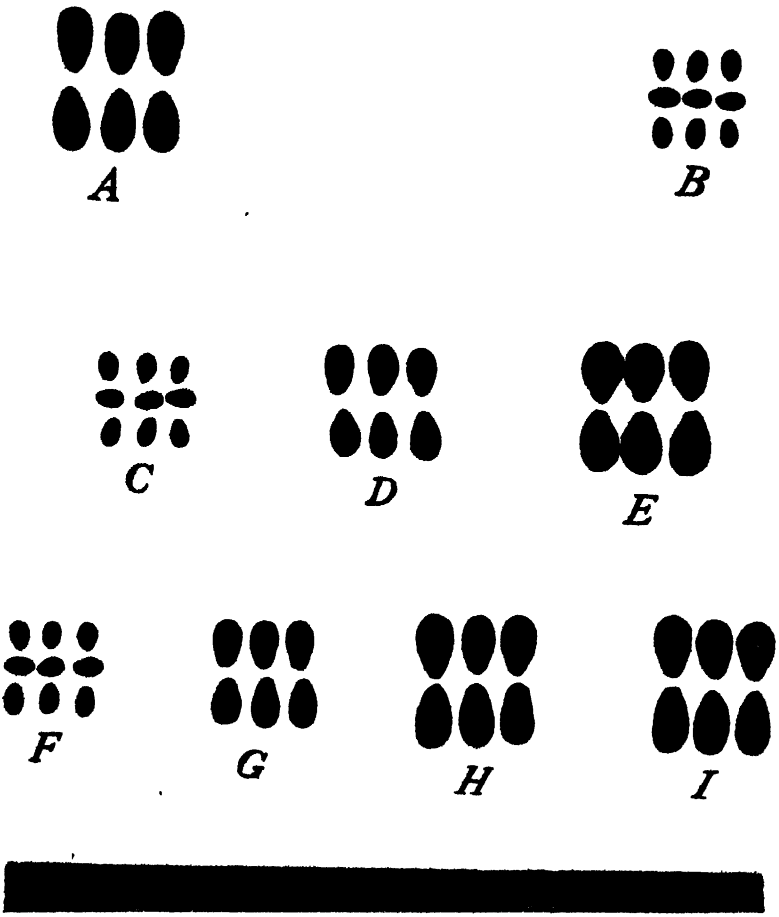

FIGURE 2.—Seed phenotypes segregating in an F_2 population: *A*, White-pink-tip long; *B*, tan short; *C*, tan short; *D*, tan medium; *E*, tan long; *F*, red short; *G*, red medium; *H*, white-tan-tip long; *I*, white-pink-tip long. For phenotypic ratio, see figure 12.

When statistical analysis required grouping for the study of inheritance of quantitative characters, such grouping was based on the size of a significant difference. Frequency tables and frequency polygons drawn from the writers' data are consequently highly useful in interpreting the Mendelian phenomena involved. Graphic treatment in frequency polygons made from seed-length data shows the

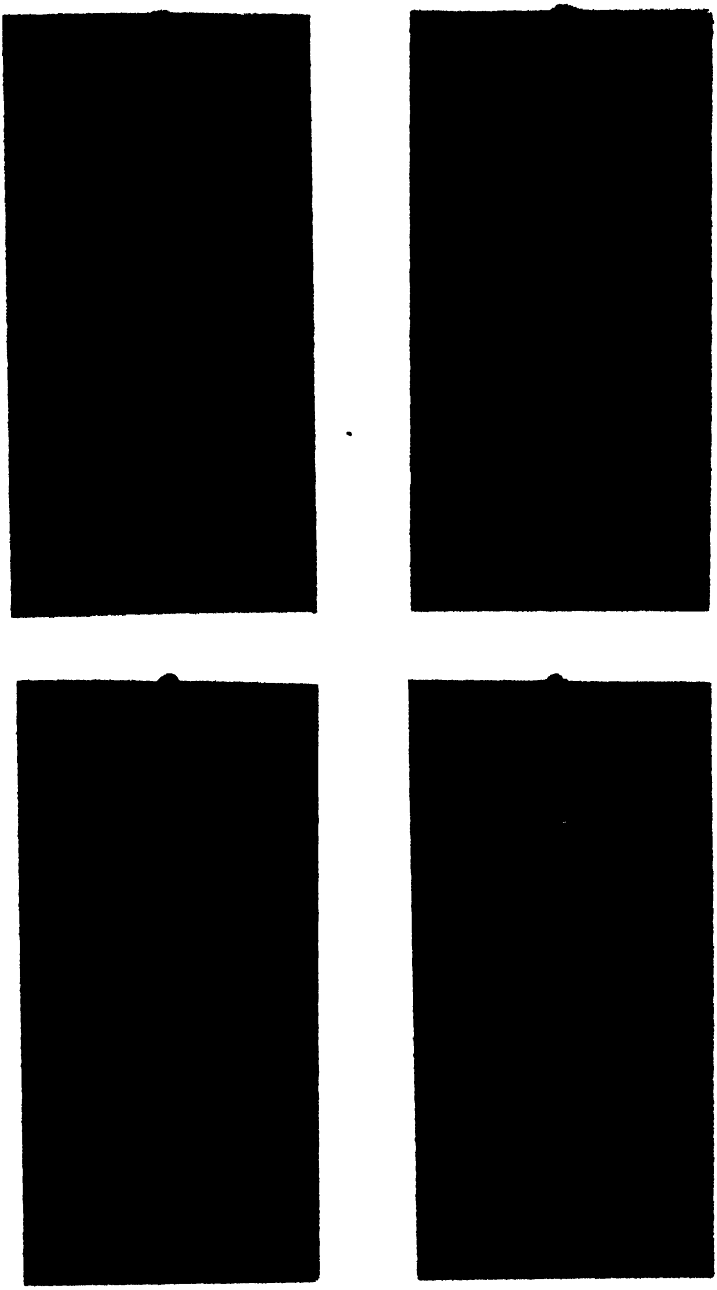

FIGURE 3. Type of envelope with cellophane window used for storing seed samples to be classified for seed-coat color.

Mendelian proportions as sharply differentiated groupings, and little difficulty exists in distinguishing one phenotype from another, except occasionally with regard to the separation of medium from long in seed-length studies.

Linkage intensities from F_2 data were estimated by two methods of Fisher (*1*), T_3 and T_4, better known as the product method and the maximum-likelihood method, respectively. The product method is the most useful in its dependability and ease of manipulation if solved by Immer's solution, which requires the use of tables for the linkage values as well as the standard errors.[3] The maximum-likelihood method is needed if any phenotype in the observed ratio contains fewer than 10 plants. The formula used for calculating the linkage value by maximum likelihood is that supplied by Owen (*7*), viz,

FIGURE 4 —Envelopes of an entire F_2 population segregating in the ratio of 27 9 12 4 : 12 seed-coat color phenotypes

$$p^2 = \frac{a-2(b+c)-d+\sqrt{(a-2(b+c)-d)^2+8dn}}{2n}.$$

INHERITANCE OF SEED LENGTH

An F_2 population of 83 plants from the cross Sun Moon and Stars × Winter Queen, when analyzed for width and length of seeds, disclosed a coefficient of correlation, $r = 0.913$, between length and width. Since length of seed is the more variable character and with respect to size presents the same kind of information as width, it was adopted for the analysis of genetic factors for seed size and for the investigation of the interactions with seed-coat color factors.

This reasoning should be sound in the present study because all varieties used as parents had long narrow seeds, but it is recognized that if any broad-seeded varieties (as found in some citron melon types) had been used, then length alone might have been misleading.

Data for seed length were available in crosses involving the combinations 6 mm. × 13 mm., 6 mm. × 10 mm., and 10 mm. × 13 mm. The three lengths 6 mm. (short), 10 mm. (medium), and 13 mm.

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[3] Dr. Immer has made available for mimeographed distribution by the Association of Southern Agricultural Workers a revision of his tables with standard errors to replace the probable errors shown in the original (*2*)

(long) represent approximate mean lengths and about the modal class in frequency distributions in segregating populations. Figures 1 and 2 show the three seed lengths segregating in two F_2 populations. Seed from a fruit of a given pure line of medium length may exhibit a larger or smaller mean length than is indicated. For instance, in Klondike, a variety of medium seed length, the seeds have an average length of 9.0 mm., yet F_1 and F_2 populations segregating from crosses to races having shorter or longer seeds usually show the mode of the medium group at approximately 10 mm., whereas the mode for this size group in backcrosses to a shorter or longer parent will be shifted in the direction of the parent's seed length. This situation is also found for long seed length, where individual inbred lines measuring 12.8 or 13.8 mm., when crossed with other seed lengths, have group modes at approximately 13 mm. in the F_2 generations, but less in backcrosses to short-length seed.

Data on observed phenotypic ratios from 17 F_2 and 19 backcross populations analyzed in this study of seed length are summarized in tables 2 and 3, and data on the 43 F_3 populations are summarized in table 4.

TABLE 2.—*Summary of F_2 and backcross generation data from four types of crosses involving seed length*

Type of cross	Phenotypes: Medium (LS)	Long (lS)	Short ($Ls+ls$)	Total	Chi-square value[1] due to indicated gene: $\chi^2 l$	$\chi^2 s$	χ^2 total
	Number	*Number*	*Number*	*Number*			
(1) F_2 medium × long	1,325	431		1,756	0.171		
(a) F_1 × long	312	301		613	.163		
(b) F_1 × medium	442			442	0		
(2) F_2 medium × short	205		77	282		0.681	
(a) F_1 × short	105		95	200		.405	
(b) F_1 × medium	172			172		0	
(3) F_2 long × short	1,067	309	411	1,787	4.613*	3.708	8.591*
(a) F_1 × long	98	80		178	1.624		
(b) F_1 × short	302		259	561		3.144	
(4) F_2 long × long		542		542	0		
(a) F_1 × long		252		252	0		

[1] A single asterisk (*) indicates significant deviation at the 19:1 level for the ratio 3L:1l.

TABLE 3.—*Analysis of seed-length data from F_2 populations from the cross short (ssLL) × long (SSll), segregating approximately 9 medium to 3 long to 4 short*

F_2 population	Phenotypes: Medium (LS)	Long (ls)	Short ($Ls+ls$)	Total	Chi-square value[1] due to indicated gene, and corresponding degrees of freedom: $\chi^2 l$	Degrees of freedom	$\chi^2 s$	Degrees of freedom
	Number	*Number*	*Number*	*Number*				
A	147	45	33	225	0.250	1	12.813**	1
B	56	31	22	109	5.245*	1	1.349	1
C	20	12	6	38	2.067	1	1.719	1
D	79	12	32	123	6.773**	1	.068	1
E	42	2	7	51	9.818**	1	3.458	1
F	139	45	53	237	.029	1	.878	1
G	317	91	141	549	1.111	1	.103	1
H	151	39	67	257	1.796	1	.105	1
I	116	32	50	198	.730	1	.000	1
Total	1,067	309	411	1,787	28.749**	9	20.493*	9
Deviation					4.613*	1	3.708	1
Heterogeneity					24.136**	8	16.785*	8

[1] A single asterisk (*) indicates significant deviation at 19:1 level; double asterisks (**) indicate significant deviation at 99:1 level.

TABLE 4.—*Summary of ratios obtained in the F_3 generation of 43 populations from the cross long (lS) × short (Ls), Peerless × Baby Delight*

Parentage in F_2	Segregation ratio in F_3	Observed frequency	Calculated frequency	χ^2 value	χ^2 value for 19 1 significance
Medium (*LlSs*)	9 medium to 3 long to 4 short.	13/26	11 6/26		
Medium (*LLSs*)	3 medium to 1 short	4/26	5 8/26		
Medium (*LlSS*)	3 medium to 1 long	6/26	5 8/26		
Medium (*LLSS*)	All medium	3/26	2 9/26	0.361	7.815
Long (*llSs*)	3 long to 1 short	5/12	8/12		
Long (*llSS*)	All long	7/12	4/12	3.375	3.841
Short (*LLss*+*llss*+*Llss*)	All short	5/5	5/5	0	
Total populations					

Table 2 clearly shows that when medium is crossed with either long or short the F_2 and backcross ratios indicate monohybrid control of seed length. The presence of the two pairs of genes *L* vs. *l* (medium vs. long) and *S* vs. *s* (medium vs. short) is confirmed by the ratios obtained from the cross long × short. The summarized F_2 populations in the latter cross offer the only instance of unsatisfactory fit between the observed and calculated proportions based on the Mendelian ratio 9 medium to 3 long to 4 short. However, backcrosses to both parents fulfill expectations.

The difficulty with the summarized F_2 of long × short is somewhat resolved by a study of the nine individual populations which comprise the summary. Table 3 shows all phenotypic ratios, together with the chi-square values traceable to each degree of freedom: χ^2_s for separation of *S* from *s*, and χ^2_l for separation of *L* from *l*. Except for population A (table 3), all the populations show expected proportions of 3 *S* to 1 *s*. In populations B, D, and E are three exceptions to the majority evidence favoring proportions of 3 *L* to 1 *l*. Examination of the frequency polygons in figures 5, 9, and 10 explains the difficulty of classifying medium (*L*) and long (*l*). Figure 5, especially, shows the distance separating the group modes in parents and F_2 to be greater between short and medium than between medium and long. The long end of the medium distribution overlaps the medium end of the long distribution, hence the difficutly of precisely separating the two phenotypes.

An F_3 generation from the cross long × short (Peerless × Baby Delight) comprised 43 populations and covered about 20 acres. Table 4 displays the seed-length data for the entire F_3 generation summarized as to F_2 parentage, the type of segregation found, and the correspondence between observed and calculated frequencies of parental genotypes for all 43 populations. The presence of five F_3 populations segregating 3 long to 1 short, together with the fact that all five short-derived populations bred true, proves that the double-recessive phenotype is short, a fact which could only be inferred from F_2 data.

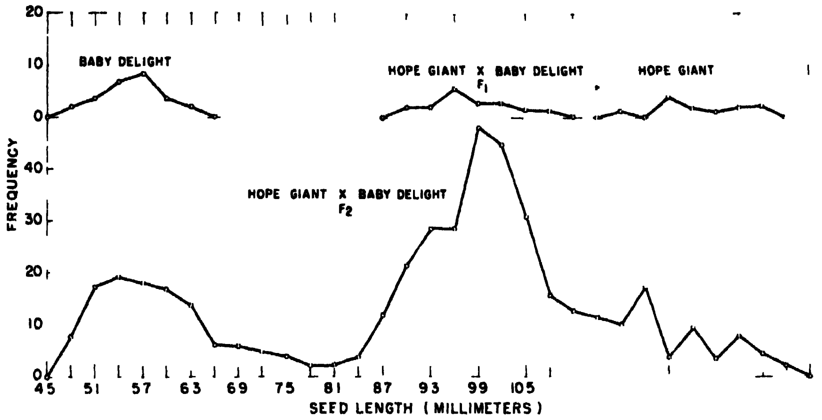

FIGURE 5.—Frequency polygons drawn from the seed-length data for Baby Delight, Hope Giant, and the F_1 and F_2 generations of the cross Hope Giant (long) × Baby Delight (short). Note the practical absence of overlap between short and medium as compared with the amount present between medium and long. The situation in F_2 parallels that existing between the parents and F_1.

In conclusion, the following gene symbols and their interaction effects as encountered in the foregoing investigation are proposed:

Medium, 10 mm., *LS*, two dominants with complementary interaction.
Long, 13 mm., *lS*, the differential effect of *l* produces long.
Short, 6 mm., *Ls*, *ls*, the differential effect of *s* produces short length in both the single- and double-recessive phenotypes.

INHERITANCE OF SEED-COAT COLOR

Investigation of seed-coat color discloses a more complex situation than that of seed size, as is expected with a larger number of phenotypes available. Phenotypic ratios from classification of 20 F_2 and 20 backcross populations are shown in tables 5, 6, 7, and 8.

The data of table 5 indicate that probably black is dominant to all other colors and patterns studied; the relationship is a single-factor dominance when black is crossed with tan or clump, but it is a two-factor dominance when black is crossed with white-tan-tip. When tan is crossed with white-tan-tip, tan is dominant by a single-factor difference; in the cross tan × white-pink-tip, tan is dominant by two factors; but when tan is crossed with clump, black is synthesized in F_1 and there is a dihybrid segregation in F_2 of 9 black to 3 tan to 3 clump to 1 white-tan-tip. When clump is crossed with white-tan-tip there is a monohybrid segregation in F_2, and when a light tan, as in Baby Delight, is crossed with darker tan, as in Thurmond Grey, there is the same color range in F_2 as in each segregating phenotype designated above as tan. The analysis will be simplified as much as possible by using the following working gene hypothesis for the genetic interactions, and by adopting the initial characterizing the first recessive gene found, according to the practice followed in investigations on *Drosophila* and *Zea mays*.

TABLE 5.—*Summary of F_2 and backcross generation data obtained from 8 types of seed-coat color crosses*

Type of cross	Phenotypes							Chi-square value[1] due to indicated gene				
	Black (*RTW*)	Tan (*RtW*)	Clump (*RTw*)	Red (*rtW*)	White tan tip (*Rtw*)	White pink tip (*rtw*)	Total	χ^2_t	χ^2_w	χ^2_{tw}	χ^2_r	χ^2_{rw}
	No	*No*	*No*	*No*	*No*	*No*	*No*					
(1) F_2 black × tan	528	180					708	0 047				
(a) F_1 × black	289						289	0				
(b) F_1 × tan	162	154					316	155				
(2) F_2 black × clump	309		98				407		0 138			
(a) F_1 × black	214						214		0			
(b) F_1 × clump	146		144				290		003			
(3) F_2 black × white tan tip	299	115	101		29		544	551	297	1 307		
(a) F_1 × black	110						110			0		
(b) F_1 × white tan tip	14	18	33		15		80	2 112	2 813	5 513*		
(4) F_2 tan × clump	623	231	234		45		1 133	214	006	12 639**		
(a) F_1 × tan	59	71					130			931		
(5) F_2 tan × white tan tip		215			53		268		3 627			
(a) F_1 × tan		254					254		0			
(b) F_1 × white tan tip		22			23		45		0			
(6) F_2 tan × white pink tip		277		96	65	18	456		10 880**		0	0 316
(a) F_1 × tan		481					481					0
(b) F_1 × white pink tip		46		23	43	32	144		174		7 563**	840
(7) F_2 tan × tan		51					51	0				
(a) F_1 × tan		67					67	0				
(b) F_1 × tan		149					149	0				
(8) F_2 white tan tip × clump			410		132		542	084				
(a) F_1 × white tan tip			55		50		105	152				
(b) F_1 × clump			117				117	000				

[1] 1 degree of freedom for each. A single asterisk (*) indicates significant deviation at 19 1 level; double asterisk (**) indicates significant deviation at 99 1 level.

TABLE 6.—*Phenotypic ratios and chi-square analysis for the F_2 populations from tan × clump seed-coat color crosses*

Population and cross[1]	Phenotypes						Chi-square value[2] due to indicated gene and corresponding degrees of freedom							
	(*DTW*), black flat	(*dTW*), black stippled	(*tW*), tan	(*Tw*), clump	(*tw*), white tan tip	Total	χ^2_d	Degrees of freedom	χ^2	Degrees of freedom	χ^2_w	Degrees of freedom	χ^2_{tw}	Degrees of freedom
	No	*No*	*No*	*No*	*No*	*No*								
1937 a	93	39	37	43	7	219	1 222	1	2 554	1	0 439	1	0 069	1
1938 a	42	19	35	28	3	127	894	1	1 388	1	003	1	7 567**	1
1938 b	92	27	49	56	13	237	137	1	114	1	1 925	1	2 363	1
1939-b	233	78	110	107	22	550	000	1	242	1	621	1	3 627	1
Total	460	163	231	234	45	1 133	2 253	4	4 298	4	2 988	4	14 252**	4
Deviation							390	1	214	1	006	1	12 639**	1
Heterogeneity							1 863	3	4 084	3	2 922	3	1 613	

[1] a = Thurmond Grey × Striped Klondike; b = Golden Honey × Baby Delight.
[2] Double asterisks (**) indicate significant deviation at 99 1 level.

TABLE 7.—*Phenotypic ratios and chi-square analysis for 3 F_2 populations from the cross white-pink-tip × tan (Peerless × Baby Delight) and 2 backcross populations from F_1 × white-pink-tip*

Cross or backcross and population	Phenotypes[1]: Tan (*RW*)	White tan tip (*Rw*)	Red (*rW*)	White-pink tip (*rw*)	Total	Chi-square value due to indicated gene, and corresponding degrees of freedom: χ^2_r	Degrees of freedom	χ^2_w	Degrees of freedom	χ^2_{rw}	Degrees of freedom
White-pink tip × tan (F_2)	*Number*	*Number*	*Number*	*Number*	*Number*						
1937 a	136	39	43	7	225	0.784	1	2.253	1	0.751	1
1937 b	76	10	35	3	124	1.817	1	13.172**	1	.516	1
1938	65	16	18	8	107	.003	1	.252	1	.757	1
Total	277	65	96	18	456	2.604	3	15.677**	3	2.024	3
Deviation						.000	1	10.880**	1	.316	1
Heterogeneity						2.604	2	4.797	2	1.708	2
F_1 × white pink tip											
1937	23 (1)	26 (1)	12 (1)	6 (1)	67	.045	1	.01	1	1.796	1
1938	23 (1)	17 (1)	11 (1)	26 (1)	77	.052	1	.636	1	4.688*	1

[1] Expected ratio shown in parentheses.

A single asterisk (*) indicates significant deviation at 19:1 level; double asterisks (**) indicate significant deviation at 99:1 level.

TABLE 8.—*Summary of data on classification for seed coat color obtained in the F_3 generation of 43 populations from the cross white-pink-tip (rtw) × tan (RtW)*

Parentage in F_2	Segregation ratio in F_3[1]	Observed frequency	Calculated frequency	χ^2 value	χ^2 value for 19:1 significance
Tan (*RrWw*)	9 tan to 3 red to 3 white tan tip to 1 white pink tip	11/29	12.889		
Tan (*RrWW*)	3 tan to 1 red	6/29	6.444		
Tan (*RRWw*)	3 tan to 1 white tan tip	3/29	6.444		
Tan (*RRWW*)	All tan	6/29	3.222	2.775	7.815
Red (*rrWw*)	3 red to 1 white pink tip	3/5	3.334		
Red (*rrWW*)	All red	2/5	1.667	0	
White tan tip (*Rrww*)	3 White tan tip to 1 white pink tip	3/6	4		
White tan tip (*RRww*)	All white tan tip	3/6	2	.188	5.991
White pink tip (*rrww*)	All white pink tip	3	3	0	0
Total		43	43.000		

[1] None of the 43 populations involved here showed, by the χ^2 test, a significant deviation from its indicated ratio.

The following genes and phenotype symbols are proposed:

RTWD black, flat (Winter Queen).

RTWd *d* differentiates a stippled surface of black dots sometimes numerous enough to be almost solid black; usually however, a tannish or reddish undercoat is visible in segregating phenotypes. *d* is apparently a specific modifier of black in the cases here considered (Klondike and Hope Giant).

RtW *t* differentiates tan, which ranges in segregating populations from dark Tuscany brown (*5, pl 7, L 11*)[4] through cacao (*5, pl 5, B 11*) as found in the variety Thurmond Grey down to light leghorn (*5, pl. 10, D 3*) as in the variety Baby Delight.

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[4] Plate and block numbers of colors given by Maerz and Paul (*5*).

RTw, *w* differentiates a pattern called "clump" because of the general tendency for the pigment in seeds of most members of this phenotype in segregating populations to clump together about the margin or in the center. In segregating populations the range of clumping runs at one extreme from uniform distribution of pigment except for a narrow line inside the margin on both surfaces, as in the Sun Moon and Stars variety (fig. 6, *B*, *a*), to the other extreme of an almost unpigmented seed except for a penciled rim or merely two dots on the hilum prominences, as in the varieties Golden Honey (fig. 6, *A*, *a*) and Northern Sweet.

Rtw, *w* and *t* together differentiate a more or less whitish seed called white-tan-tip, sometimes with a slight tannish shading on the margin, but the shading is always present at the hilum end (Pride of Muscatine, Gandy, Chilean, and some lines of Peerless).

rtW, *r* and *t* together differentiate a red- or reddish-orange colored seed ranging in segregating populations from fez red (*5, pl. 5, L 4*) to powder pink (*5, pl. 3, E 7*). (Not used as a parent but segregating from Peerless × Baby Delight, *rtw*×*RtW*.)

rtw, *r*, *t*, and *w* together produce a decidedly white seed with slight pink color at the hilum end, called white-pink-tip, and sometimes a trace of pink along the margin in segregating populations (Peerless).

Table 5 shows that with four exceptions the observed phenotypic ratios conform to expectation based on the foregoing hypothesis of three genes for color. The right-hand side of the table shows the portion of chi square that is contributed by each degree of freedom. The four poor fits are thus traced to the gene interactions responsible.

The first poor fit, black×white-tan-tip backcrossed to white-tan-tip, is due to the interaction between *T* and *W*, although the difficulty in this backcross is peculiar to it because the χ^2_{tw} from the F_2 generation of the same cross shows no disturbance at this point. The second poor fit, in the F_2 of tan×clump, is also due to the interaction of *T* and *W*. In table 6 the four populations resulting from this cross are orthogonalized separately, and show that the difficulty between *T* and *W* is peculiar to the one population of Thurmond Grey × Striped Klondike grown in 1938. The three other populations segregate according to hypothesis. Hence it may be concluded that no genetic linkage occurs between the genes *T* and *W*.

The third and fourth cases of unsatisfactory fit occur in the F_2 progenies of tan×white-pink-tip and the backcross of this F_1 to white-pink-tip. In the F_2 populations χ^2_w is involved; in the backcross χ^2_r is involved. Table 7 shows that the 1937-a population was a single F_2 generation of 225 plants, whereas the 1937 b population was an aggregate of three smaller F_2 populations of 25, 73, and 26 plants, respectively. The failure of the combined three populations to give an acceptable χ^2_w when totaled is ascribed to the inadequate sizes of the populations individually and collectively. (N. B. The seed-length gene *L* has already been seen in the foregoing section to be irregular in this same cross.) In the first backcross population it was assumed that F_1 was heterozygous for the *r* allelomorphs; hence a 3:3:1:1 ratio in this backcross gives a satisfactory χ^2 estimate. In the second backcross population an excessive proportion of white-pink-tip at the expense of red is responsible for the disturbed interaction between the *r* and *w* genes.

The cross white-pink-tip × tan (Peerless × Baby Delight) recovers the parental phenotypes as double recessive and double dominant, respectively, in a dihybrid segregation, and the two single recessives are white-tan-tip and red. When one of these single recessives,

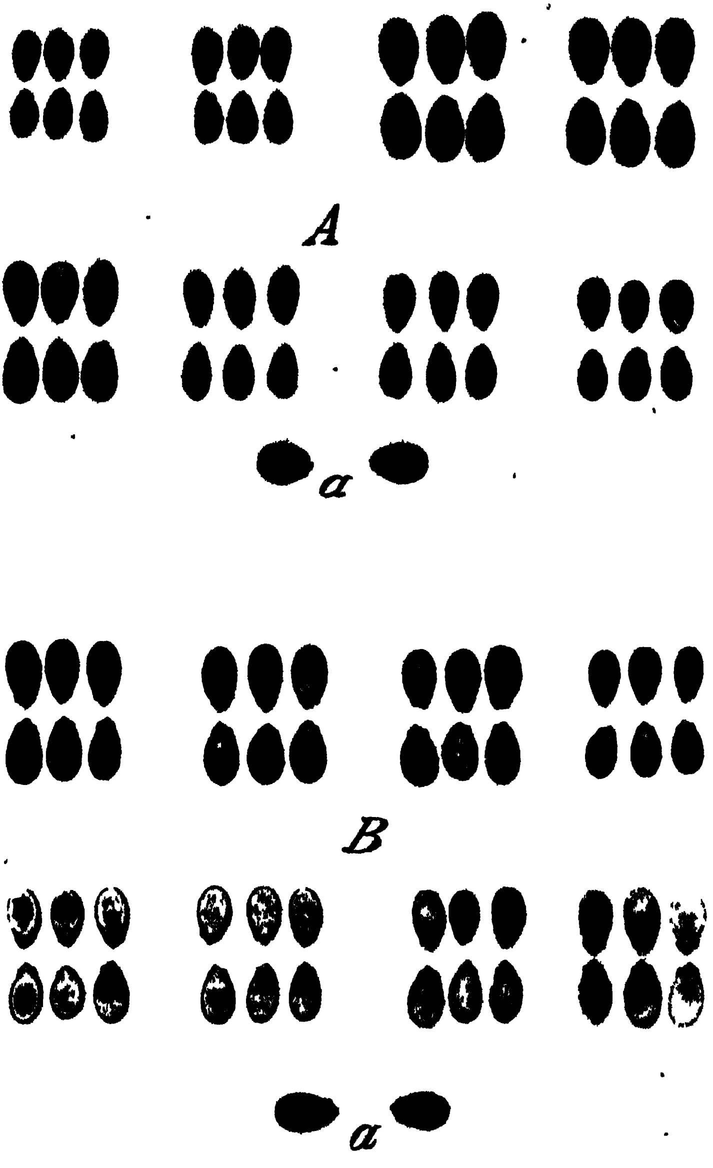

FIGURE 6.—Range of pigmentation displayed in clump phenotype when segregating in the F_2 populations of two crosses. *A*, Golden Honey (restricted clump) × Baby Delight (tan); *a*, sample from clump parent. *B*, Sun Moon and Stars (full clump) × Klondike (black); *a*, sample from clump parent.

white-tan-tip, is crossed with black (Pride of Muscatine × Klondike), the two parental forms are also recovered as double recessive and double dominant, respectively, while the two single recessives are tan and clump. The inference arises that in these two crosses, instead of four different genes, there are only three. All other crosses in table 5 confirm this hypothesis. Accordingly, the three genes *RTW* are postulated as the chief elements in a gene-interaction system in which simultaneous segregation of *R* and *T* has not yet been obtained, while *RW* and *TW* are shown to be inherited independently of each other. A fourth gene, *D* (table 6), operates as a modifier of black, *RTW*, and the simple allelic relations of *D* and *d* are demonstrated by four separate F_2 populations.

In Golden Honey the clump background has almost disappeared except for a marginal band and shading on the hilum end as shown in figure 6, *A*, *a*. There is no question that the recessive allel *w* is present with *T*, and that when it is crossed with tan in Golden Honey × Baby Delight the same full range of the pattern is displayed in three-sixteenths of the F_2 population as in the two other crosses, Sun Moon and Stars (white-tan-tip) × Klondike (black) and Thurmond Grey (tan) × Striped Klondike (clump). Figure 6 shows the range of expression of the phenotype clump in F_2 populations of the crosses (*A*) Golden Honey × Baby Delight and (*B*) Sun Moon and Stars (white-tan-tip race) × Klondike (black).

In order to test further the validity of the color-genes hypothesis adopted from the foregoing F_2 and backcross studies, an F_3 generation from the cross white-pink-tip × tan was grown in the summer of 1939. A summary of the data obtained from F_3 classification for seed-coat color appears in table 8, and is arranged to show F_2 parentage, the type of segregation found, and the correspondence between observed and calculated frequencies. Out of 29 populations derived from tan-colored parents, 6 proved to be from *RRWW* and 3 from *RRWw* parents, thus constituting a slight but not serious reversal of expectation; the remaining 34 populations shown in the table segregated in close agreement with the Mendelian proportions expected from the foregoing hypothesis.

RELATION BETWEEN SEED-LENGTH AND SEED-COAT COLOR GENES

Figures 7 and 8 show that when the allels *T* (black) and *t* (tan) interact with *L* and *l* (medium and long) or *S* and *s* (medium and short) there is independent assortment of all genes concerned. The two figures graphically demonstrate the complete dominance of black over tan, medium over large, and medium over small, as well as the independent assortment of the three pairs of genes into two 9 : 3 : 3 : 1 ratios in F_2, 1 : 1 : 1 : 1 in backcrosses to the double-recessive parent, and all black medium in backcrosses to the triple-dominant parent.

The first indication that linkage existed between quantitative and qualitative genes for seed characters arose from the observation in 1935 that small white seeds were rare in crosses expected to produce them. The approximate linkage value calculated from the 1935 cross of large white-pink-tip × small tan (Peerless × Baby Delight) was also obtained in 1937 from the cross large white-tan-tip × medium black (Pride of Muscatine × Klondike). The observation that white-tan-tip was involved in linkage with white-pink-tip in the first cross and that white-tan-tip and clump were involved in the other cross suggested that the same recessive gene *w* helps determine the two white phenotypes and clump.

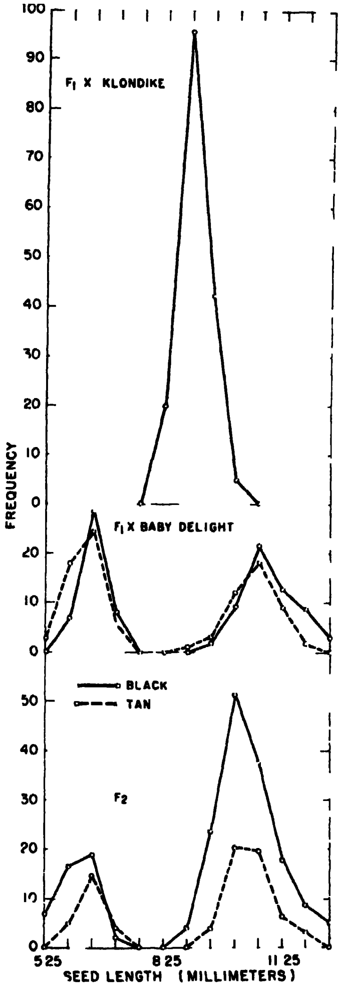

FIGURE 7.– Klondike × Baby Delight (black medium × tan short): F_2 and backcrosses to both parents, segregating for two color and two length phenotypes- black, tan, medium, and short.

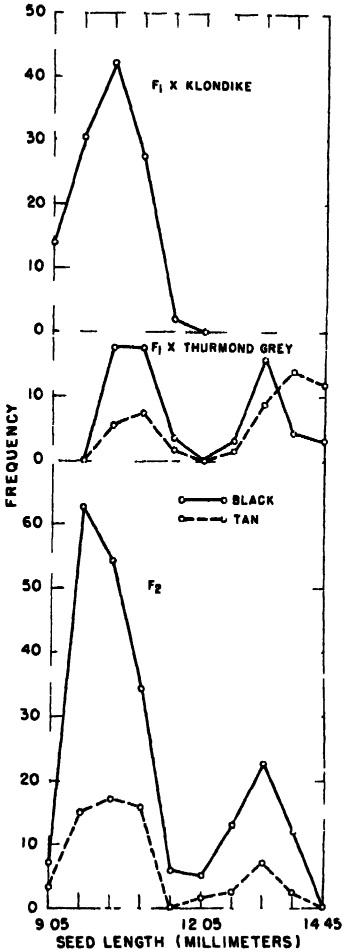

FIGURE 8. Klondike × Thurmond Grey (black medium × tan long): F_2 and backcrosses to both parents, segregating for two color and two length phenotypes—black, tan, medium, and long. Independent assortment.

In the study of the interactions of the four color and two seed-length genes by orthogonal chi-square values, no population available included at one time more than four of the six. The two crosses offer-ing quadrihybrid segregations (table 9) were Golden Honey × Baby Delight, segregating for *LST* and *W*, and Peerless × Baby Delight segregating for *LSR* and *W* (see figs. 12 and 13). Several crosses segregated for *LT* and *W*, but they will not be discussed since they add nothing to the information from table 9 or from previous tables. Since the allels *D* and *d* operate only on the black phenotype, gene interaction is limited to *D* and *L* or *D* and *S*, the effects of which are seen in tables 10 and 11.

TABLE 9.—*Sources of chi-square from the 9 possible single and double genic interactions in the 2 F₂ populations Golden Honey × Baby Delight (LSTw × LstW) and Peerless × Baby Delight (LSru × LsRW)*

Golden Honey × Baby Delight: Phenotypes: Gene symbol	Number	Chi-square value[1]	Peerless × Baby Delight: Phenotypes: Gene symbol	Number	Chi-square value[1]
LSTW	293	χ = 0.462	*LSRW*	178	χ = 0.011
LSTw	48	χ = 0.646	*LSRw*	18	χ = 6.707**
LStW	99	χ = 0.004	*LSrW*	74	χ = 0
LStw	8	χ = 0.031	*LSrw*	7	χ = 9.793**
L+lsTW	91		*L+lsRW*	73	
L+lsTw	47	χ = 0.282	*L+lsRw*	2	χ = 3.111
L+lstW	46	χ = 147.260**	*L+lsrW*	15	χ = 105.816**
L+lstw	2		*L+lsrw*	0	
lSTW	43		*lSRW*	28	
lSTw	68	χ = 0.012	*lSRw*	45	χ = 2.135
lStW	14	χ = 0.062	*lSrW*	5	χ = 9.839**
lStw	26	χ^2 = 6.015*	*lSrw*	13	χ = 0.024
Total	785	χ = 151.877**	Total	458	χ^2 = 138.429**

[1] One degree of freedom for each. A single asterisk (*) indicates significant deviation at 19:1 level; double asterisks (**) indicate significant deviation at 99:1 level.

TABLE 10.—*Frequency distribution of phenotypes and chi-square analysis of gene interaction between stippling and long seed factors in (Hope Giant × Baby Delight) × Hope Giant*

Cross	Phenotypes (all black): Flat medium (*DL*)	Flat long (*Dl*)	Stippled medium (*dL*)	Stippled long (*dl*)	Total	Chi-square value[1] due to indicated gene and corresponding degrees of freedom: χ^2_d	Degrees of freedom	χ^2_l	Degrees of freedom	χ^2_{dl}	Degrees of freedom
	Number	*Number*	*Number*	*Number*	*Number*						
WBC 19a	66	54	74	52	246	0.102	1	4.427*	1	0.260	1
WBC 19c	41	28	28	30	127	.787	1	.787	1	1.543	1
Total	107	82	102	82	373	.889	2	5.214	2	1.803	2
Deviation						.043	1	5.190*	1	.043	1
Heterogeneity						.846	1	.024	1	1.760	1

[1] A single asterisk (*) indicates significant deviation at 19:1 level.

TABLE 11.—*Chi-square analysis of gene interaction between stippling and long- and short-seed factors, using only relevant data from F_2 populations also segregating for phenotypes that are omitted here*

Phenotypes: Gene symbol	Phenotypes: Number	Chi-square value [1]	Degree of freedom	Phenotypes: Gene symbol	Phenotypes: Number	Chi-square value [1]	Degree of freedom
DL	237	$\chi^2_D = 1.252$	1	*DS*	79	$\chi^2_D = 0.003$	1
Dl	57	$\chi^2_l = 9.779^{**}$	1	*Ds*	13	$\chi^2_S = 12.160^{**}$	1
dL	91	$\chi^2_{Dl} = 1.089$	1	*dS*	30	$\chi^2_{DS} = 1.518$	1
dl	17			*ds*	1		
Total	402	12.120^{**}	3	Total	123	13.681^{**}	3

[1] Double asterisks (**) indicate significant deviation at 99.1 level.

In the Golden Honey × Baby Delight population, out of 12 phenotypes (therefore 11 degrees of freedom), only the 9 single and double gene interaction degrees shown in table 9 are useful. The two triple gene interaction degrees remaining give no linkage information and may be omitted. The data show a strong presumption of linkage ($\chi^2_{LW} = 147.260$, 1 degree of freedom) for the genes *L* and *W*, and a weak one for *T* and *W* ($\chi^2_{TW} = 6.065$). This last-named value ($\chi^2_{TW} = 6.065$) has already been analyzed (table 6) for the two sections of the population grown in 1938 and 1939, respectively. The two chi-square values in question are 2.363 and 3.627, neither of which is significant, although when the two sections are summated to make one population, as in table 8, the individual shortages of white-tan-tip plants accumulate to give an increased chi-square value.

Interaction of *L* and *S* cannot be studied in this or any other cross because in the 9:3:4 ratio present there are but 2 degrees of freedom, viz, *L* vs. *l*, and *S* vs. *s*, and there is no way of orthogonalizing the *L* vs. *S* interaction. However, if *L* and *S* were linked, then *S* and *W* would also be linked, since linkage exists between *L* and *W* (see data to follow).

The Peerless × Baby Delight cross will give little reliable information on the genes *S* and *W* or their interaction, because the single-gene chi-square values are each statistically significant. $\chi^2_{lw} = 105.816$ indicates the second strong presumption of coupling linkage between *L* and *W* in corroboration of the preceding cross evidence. A second significant chi square, $\chi^2_{sw} = 9.899$, 1 degree of freedom, probably results from that given above. χ^2_{sr} and χ^2_{rw} are nonsignificant; consequently the chances are against the presence of any linkages in this series except the possible but yet untried combination *T* and *R*, and the highly probable *L* and *W*, to be evaluated later.

A graphic portrayal of the different types of factor interaction between these color and length genes is presented in the series of frequency polygons (figs. 7–13). All crosses not represented graphically are in agreement with the interpretations offered.

The gene interactions involving *D* with *L* from the backcross (black stippled long × tan flat short) × black stippled long are shown in table 10. Data are given for the only two backcross populations where interaction of *D* with *L* can be followed without interference from other genes. Both populations are from the same parentage and both give the information that no linkage relations exist between genes *L* and *D*. No crosses are available showing only the interaction of *D* with *S*, since that would require an as yet unknown parent having stippled black short seeds. However, it is possible to approximate the relations of *D* with *L* and *S* by omitting irrelevant data from more complex F_2 segregations. These approximations are given in table 11 and show that within such limits no linkage relations exist between *D* with either *L* or *S*.

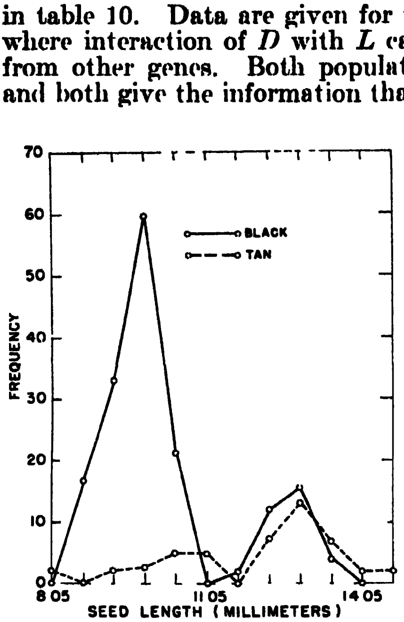

FIGURE 9.—Klondike × Sun Moon and Stars (black medium × clump long) F_2 population showing coupling-phase linkage between *L* and *W*.

The only gene interaction manifesting clear-cut demonstrations of linkage is that between *L* and *W*, the effects of which are graphically shown in figures 9 to 13. In table 12 are given all relevant data on 10 F_2 populations in the coupling phase, 2 F_2 populations in repulsion, and 7 backcrosses, together with estimates of linkage intensity.

The populations in coupling and backcross to the double-recessive parent are always most satisfactory for estimating linkage intensities, as Fisher (*1*) and Mather (*6*) have shown; consequently the coupling value 19.3±1.1 percent, estimated by the product method, and the value 15.8±1.2 percent, obtained by direct calculation of cross-overs in the backcross, give a more reliable estimate than the value 24.7±4.9 percent, estimated by the maximum-likelihood method from the repulsion populations.

TABLE 12.—*Analysis of sources of chi-square and estimate of linkage value for L and W in populations segregating for seed length (medium, L, vs. large, l), and seed color (black or tan, W, vs. white or clump, w), in 10 F_2 coupling, 2 F_2 repulsion, and 7 backcross populations*

Type of cross	Phenotypes					Linkage value	Chi-square value due to indicated gene[1]		
	LW	*Lw*	*lW*	*lw*	Total		χ^2 *l*	χ^2 *w*	χ^2 *lw*
	Number	*Number*	*Number*	*Number*	*Number*				
Coupling	1,031	124	140	240	1,535	19.3±1.1	0.006	1.355	416.591
Repulsion[3]	195	77	71	4	347	24.7±4.9	1.945	.424	16.089
Backcross	362	74	67	389	892	15.8±1.2	.405	1.221	414.422

[1] Each χ^2 has 1-degree of freedom
[2] Significance level 19 1=3.841, 99 1=6.635.
[3] By maximum-likelihood method.

However, Mather (*6, p. 63*) shows that a completely classified F_2 population "contains twice as much information about the recombination fraction" as a backcross. Consequently, the fact that the 43 F$_3$ populations indicate the full genotypes of their F$_2$ parents provides a degree of precision not available in the data of table 12. In table 13, therefore, is seen the distribution by phenotype symbols for *L* and *W* of the 43 parent plants of the F$_3$ generation. Calculation

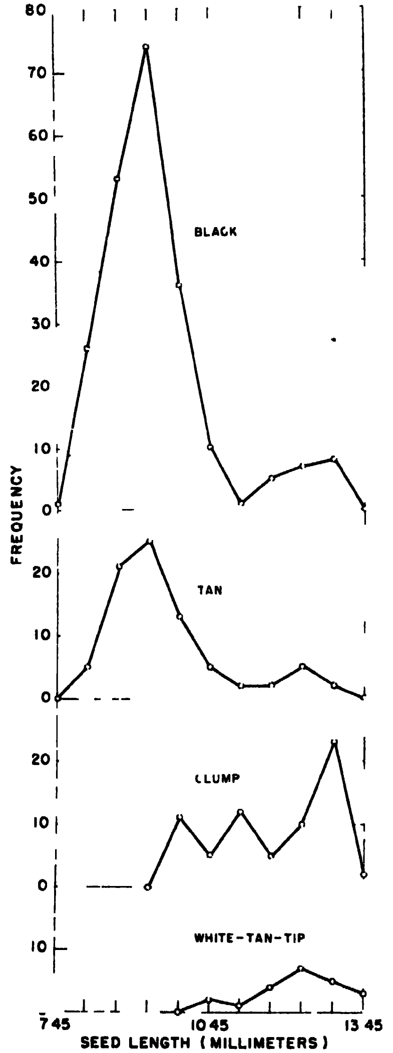

FIGURE 10. - Pride of Muscatine × Klondike (white-tan-tip long × black medium). F$_2$ population segregating in four color phenotypes, black, tan, clump, and white-tan-tip, and two length phenotypes, medium and long. Coupling linkage between *L* and *W*.

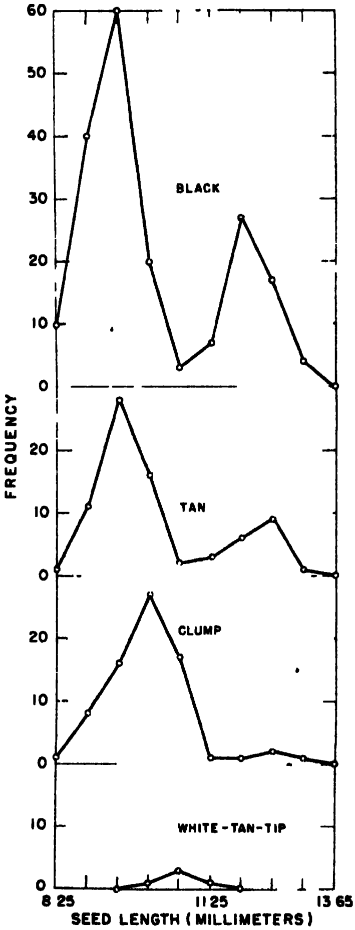

FIGURE 11.- Striped Klondike × Thurmond Grey (clump medium × tan long): F$_2$ population segregating in same phenotypes as in figure 10, but with repulsion linkage between *L* and *W*.

of the linkage value by the maximum-likelihood method indicates a coupling linkage value of 21.52±7.24 percent, which falls within the range indicated by the data of table 12.

TABLE 13.—*Analysis of the distribution by phenotype symbols of the identified genotypes of 43 parent plants of the F_1 generation from the cross LW × lw (tan medium × white-pink-tip long)*

Phenotype	Frequency	Chi-square value[1]	Linkage value
			Percent
LW	29	$\chi^2_l = 0.070$	21.52±7.24
Lw	2	$\chi^2_w = 0.628$	
lW	6	$\chi^2_i = 6.721^{**}$	
lw	6		
Total	43		

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[1] Double asterisks (**) indicate significant deviation at 99:1 level.

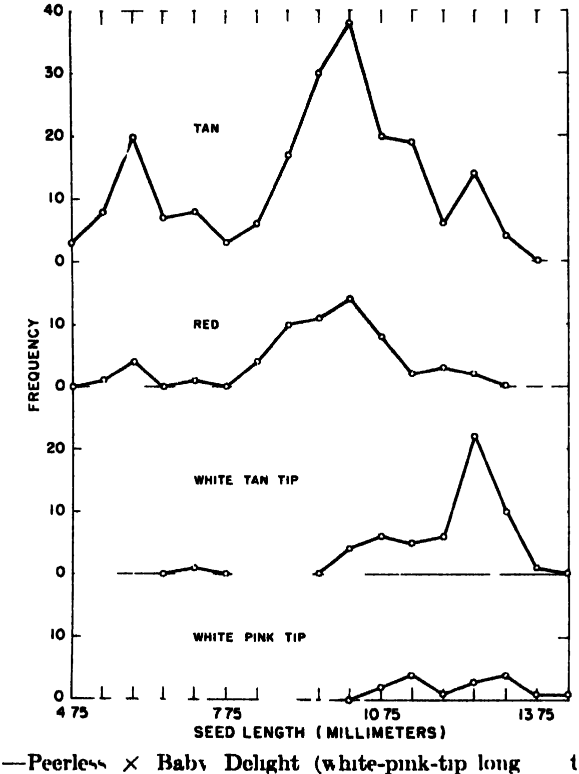

FIGURE 12.—Peerless × Baby Delight (white-pink-tip long × tan short) F_2 population segregating in four color phenotypes, tan, red, white-tan-tip, and white-pink-tip, and three length phenotypes, short, medium, and long. Coupling linkage between *L* and *W*.

DISCUSSION

Weetman (*9*) studied inheritance of seed weight (units of 25 seeds per fruit) of F_1, F_2, and backcross populations for the cross light (1.22±0.01 gm.) × heavy (2.60±0.05 gm.). In reciprocal crosses the

F_1 mode varied a little, but was always closer to the mode of the lighter than the heavier parent. F_2 and backcross distributions were bimodal, suggesting segregations of 3 light to 1 heavy for F_2, all light for backcrosses to the light parent, and 1 light to 1 heavy for backcrosses to the heavy parent. Despite significant deviations between observed and calculated ratios, these data indicate that Weetman had parental races corresponding to the medium and long-seeded phenotypes of the present investigation. The writers obtained good fits between observed and calculated ratios because all phenotypes had long, narrow seeds, hence variation was mainly in length. There are biotypes in *Citrullus vulgaris*, however, with relatively broad seeds, and crosses between this and the long narrow phenotype would probably segregate for more genes than *L* and *S*. The poor fit observed by Weetman may have arisen from the presence of seed-shape genes in addition to seed-length genes.

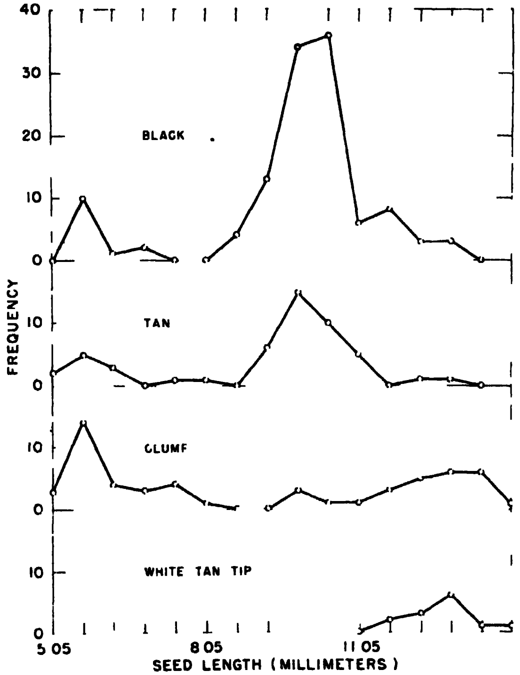

FIGURE 13. Golden Honey × Baby Delight (clump long × tan short) F_2 population segregating in four color phenotypes, black, tan, clump, and white-tan-tip, and three length phenotypes, short, medium, and long. Coupling linkage between *L* and *W*.

In parent varieties there are at least two extreme kinds of clump and tan seed, which, on crossing with another phenotype, present the same shade range in the segregating clump or tan phenotype found in F_2. This shade range within the same phenotype in segregating populations is not caused entirely by accompanying dominant genes of the triple interaction system, for the two original parent varieties Golden Honey and Sun Moon and Stars (fig. 6) were certainly not segregating. The range from light tan identified from Maerz and

Paul (*5*) as leghorn (*pl. 10, D 3*) to dark brown (*Tuscany, pl. 7, L 11*), runs parallel to the shade range of the poultry breed called Brown Leghorn. In the case of poultry the predominant shade is leghorn, as the breed name indicates, but much darker shades of brown are frequently found within the breed, and upon crossing with other phenotypes give the same segregation range as the lighter leghorn shade.

In the early stages of these investigations the extremely reduced clump phenotype of Golden Honey was thought to be white rather than clump (*8*), thus causing some confusion in classifying for color.

Red in segregating populations is another phenotype giving a wide range of shades from fez red to powder pink, as illustrated by Maerz and Paul (*5*), and no doubt different investigators will call this phenotype by different names, such as reddish brown, orange, or red. The recognition of the extent of the shade range comprising a given phenotype is facilitated by using envelopes with cellophane windows when classifying Mendelian populations. For the sake of future genetic studies of watermelon it is important that investigators know they are dealing with a given genotype no matter under what names it may appear. If the range of shading found within a given phenotype is the same from cross to cross and appears repeatedly in the approximate proportions expected by a working hypothesis, then the existence of black, clump, tan, red, white-tan-tip, and white-pink-tip as Mendelian phenotypes is established. Moreover, all the phenotypes but black and clump appear as expected by gene analysis right into F_3, and these fit so adequately into the interaction system here developed, besides confirming the findings of other investigators, as to render further investigations of these two unnecessary.

Kanda (*3*) in 1931 published a preliminary report on inheritance studies in seed-coat color, in which he presented detailed studies of only 2 crosses, one evidently involving the same pair of factors here called black flat and black dotted (*Dd*), which was carried to F_3, and the other postulated as segregating for 4 pairs of factors including *Dd*. About 80 additional crosses reported had not gone beyond F_1. The cross reported to segregate for 4 pairs of factors for seed-coat color comprised 123 plants in F_2 and was carried no further, despite the fact that an adequate analysis required a minimum of 1,024 plants to include the quadruple recessive at 5 percent probability and between 1,590 and 1,855 plants to include it at 1-percent probability. When Kanda's F_2 phenotypic ratio is subjected to a test for goodness of fit, as in table 14, the chi-square value obtained is 12.636, with 5-percent significance at 9.488 and 1-percent significance at 13.277. If his phenotypes are compared with those obtained in the present material, a closer agreement can be reached by assuming only 3 pairs of factors, which will produce a chi-square value of 9.010, with the same levels of significance as given above. In the suggested postulation *T* and *D* are certain (according to the writers' hypothesis) to be included, and *R* is suggested as the third gene. Even if this is an oversimplification, it fits the situations already known, provided Kanda's "brown clear" phenotype comprises the range observed for the writers' "red" phenotype.

Weetman's (*9*) study of seed-color inheritance supports the writers' interpretations when allowance is made for inevitable personal bias in the choice of names for fluctuating shades of color. For example, Weetman's description of Japan 4 seed color as "medium brown with fine black dots" is the writers' dotted black, and his Long Iowa Belle, described as "light tan with peripheral black band," is the writers' clump (see fig. 6). Description of the F_1 between them as "brown nonbanded * * * much darker, as the fine black dots were more numerous" is an accurate description of the writers' F_1 Thurmond Grey × Striped Klondike, or Golden Honey × Baby Delight. F_2 and the backcross to Long Iowa Belle (clump) gave the expected segregation of 3:1 and 1:1, respectively, and the backcross to Japan 4 (black) was "all brown nonbanded" as expected.

TABLE 14. *Kanda's F_2 population with gene analysis given, and a proposed revision in the light of the present investigation*

Observed frequency		Kanda analysis		Suggested revision	
Phenotype	Number	Expectation	Postulation	Expectation	Postulation
Black flat	45	38.92	*MHK*	51.9	*RTD*
Black dotted	21	13.96	*MHk*	17.3	*RTd*
Brown knob	21	17.29	*Mh* (*K* or *k*)	23.1	*rT* (*D* or *d*)
Brown clear	15	23.05	*m* (*Hh*, *Kk*)	7.7	*rt* (*D* or *d*)
Tan	21	30.75	*aM* (etc.)	23.1	*Rt* (*D* or *d*)
Total	123	122.97		123.1	
χ^2 value[1]		12.636		9.010	

[1] For 5 percent probability = 9.488, for 1 percent probability = 13.277

In Weetman's second cross, involving Japan 6 and Long Iowa Belle, the female parent is referred to as "reddish brown" and the F_1 between them as "black," doubtless the writers' flat black. "Reddish brown" in this case is probably the writers' dark tan, which when crossed to clump produced 9 black, 3 "yellowish to reddish brown" (tan), 3 "light tan with black bands" (clump), and 1 "tan quite variable" (white-tan-tip). The backcrosses to both parents segregated as expected for these phenotypes.

In Weetman's third cross, Japan 6 and China 23, the latter had a clump phenotype, and F_2 (expected to segregate 9 : 3 : 3 : 1 as above) was classified as 9 "dark brown or black," 4 "reddish or yellowish brown," and 3 "tan banded." The backcross to the tan parent gave 1 black and 1 tan. In F_2 the 4/16 class was obviously a difficult one to classify and the double-recessive phenotype could not be identified with complete assurance.

The investigations reported by the present writers substantiate the published reports from other sources, and by adding new data coordinate the information now available on form and size of seed and on seven seed-coat color phenotypes into a unified system somewhat resembling the classic system of aleurone colors occurring in *Zea mays* L.

SUMMARY

The three seed-length phenotypes used in this study—short (average about 6 mm.), medium (average about 10 mm.), and long (average about 13 mm.)—behave with respect to each other as though belonging in a dihybrid F_2 segregation of 9 medium (*LS*) to 3 long (*lS*) to 4 short (*Ls* and *ls*).

Seed-coat color phenotypes of parent biotypes used in this study are black, clump, tan, white-tan-tip, and white-pink-tip. Of 10 possible combinations 7 have been made. Analysis of F_2, F_3, and backcross populations indicates that the colors and patterns of this series are determined by the interaction of a system of 3 main genes *RTW* and 1 specific modifier *D* operating on black alone. Of the 8 possible phenotypes from the 3 main genes *RTW*, 6 are indicated, *RTW* (black), *RT*w (clump), *RtW* (tan), *Rtw* (white-tan-tip), *rtW* (red), and *rtw* (white-pink-tip). *RTWd* restricts the black pigment to dots more or less uniformly distributed.

There is no evidence of linkage within groups of the length (*LS*) or color (*DRTW*) genes, since all possible combinations have been analyzed except *TR*. But between groups, the genes *L* and *W* display coupling linkage of 19.3 ± 1.1 in F_2 and 15.8 ± 1.2 in backcross, 24.7 ± 4.9 repulsion linkage in F_2, and 21.52 ± 7.24 coupling linkage among the completely identified parents of an F_3 generation.

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INCREASED SIZE AND NICOTINE PRODUCTION IN SELECTIONS FROM INTRASPECIFIC HYBRIDS OF NICOTIANA RUSTICA [1]

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INTRODUCTION

At present nicotine is obtained from leaf stems and low-grade or scrap-leaf material of *Nicotiana tabacum* L. as a byproduct of the tobacco industry. However, with the possibility of an increasing demand for nicotine, because of its wider use in insecticides and for other purposes, there may be need for varieties of tobacco that can be grown profitably for the nicotine alone.

As early as 1911 *Nicotiana rustica* L. was shown [2] to have a higher content of nicotine than *N. tabacum*, and it was suggested at that time as a logical species to use as a source of this alkaloid.

For a number of years many different varieties and introduced strains of *Nicotiana rustica* have been grown by the Division of Tobacco Investigations and tested for yield and alkaloidal content. Types with the highest yields of nicotine were selected and continued each season. It was considered, however, that even among the best of these there was need for improvement of plant characteristics and especially for larger size.

The late Otto Olson, of this Division, working in cooperation with the Pennsylvania Agricultural Experiment Station, crossed two favorable varieties of *Nicotiana rustica* some years ago, and selections from his hybrids have also been continued.

With these sources of material the investigations reported herein were begun in 1936. Their purpose was to combine the best size characteristics to obtain a larger *Nicotiana rustica* giving increased yield of nicotine.

MATERIALS AND METHODS

Two inbred varieties from introduced seed lots and one of the best Olson selections were used as parents for crosses. They are described below.

(1) *Nicotiana rustica* var. *brasilia* Schrank, strain 34753 (fig. 1, *A*) Seed was obtained from A. Splendore, Scafati, Italy, in 1913, and was noted as coming originally from Brazil.

(2) *Nicotiana rustica* tall type (fig. 1, *B*). Seeds were obtained in 1926 from the Cambridge Botanical Garden, Cambridge, England.

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[1] Received for publication March 4, 1941.

[2] GARRAD, G. H. THE GROWING OF TOBACCO FOR NICOTINE EXTRACTION. Southeast. Agr. Col. Jour. 20: 367-393. 1911

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They were not labeled with any varietal designation and have been called simply tall type. The strain is probably nearest to *N. rustica* var. *scabra* Cav.

(3) *Nicotiana rustica* Olson 68 (fig. 1, *C*). This line was selected from a cross between *N. rustica* var. Winnebago and *N. rustica* var. *brasilia* No. 23. It was continued by inbreeding through a number of generations until in 1938–39 the F_{10} and F_{11} were grown.

All plants in these experiments were grown at the Arlington Experiment Farm, Arlington, Va. They were placed 18 inches apart in 1-meter rows so that there were 8,800 to the acre. Shortly before planting time, 4-8-4 fertilizer was distributed uniformly in the drill at the rate of 800 pounds per acre. The soil was Keyport silt loam, a heavy, claylike type, and was approximately uniform throughout the plots used.

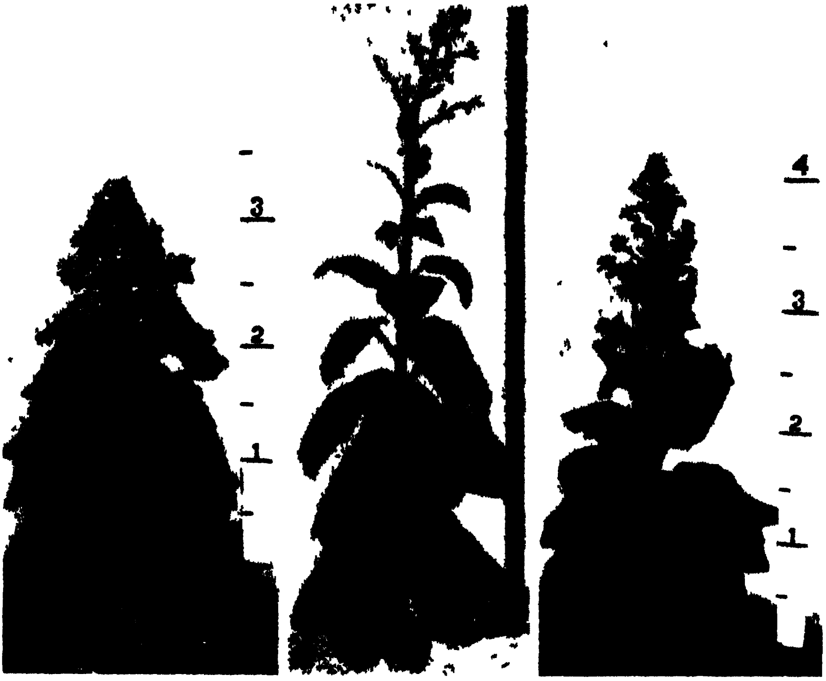

FIGURE 1.—Parental types of *Nicotiana rustica* used in crosses *A*, Variety *brasilia*, strain 34753; *B*, tall type; *C*, Olson 68.

Measurements on plant height, number of leaves, and length and width of the largest leaf were made soon after the onset of flowering. The best plants were bagged, and about 28 days later their leaves were picked and hung in the curing barn to dry.

Following the drying, or curing, selected leaf samples were weighed and ground into a fine powder preparatory to chemical analysis. A modification of the Keller method, developed in the Division of Tobacco Investigations, was used to determine the nicotine content.[3]

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[3] GARNER, W. W., BACON, C. W., BOWLING, J. D., and BROWN, D. E. THE NITROGEN NUTRITION OF TOBACCO. U. S. Dept. Agr. Tech. Bul. 414, 78 pp., illus. 1934.

RESULTS ON INCREASING SIZE

PARENTS, F_1'S, AND F_2'S

Average measurements on the three parental types used are arranged in table 1. The year during which each family was grown is prefixed to its number; e. g., 37190 in 1937, 38179 in 1938. It is evident that pronounced yearly fluctuations occurred and must be taken into account when making comparisons between parents and hybrids.

Table 1. *Means of measurements on parental strains of Nicotiana rustica and on F_1 and F_2 generation families resulting from crosses between them*

Parent or cross	Generation	Family No	Number of individuals	Average plant height	Average number of leaves	Average length of largest leaf	Average width of largest leaf
				Inches		*Inches*	*Inches*
Variety *brasilia*, 34753	P_1	37190	38	28 5±0 31	19 5±0 31	13 2±0 21	10 4±0 23
	do	38179	20	29 9± 54	24 5± 82	13 1± 15	11 5± 14
	do	39232	5	14 4±1 78	11 8±2 35	9 7± 41	10 0± 65
Tall type	do	37192	68	38 4± 32	18 9± 29	12 2± 23	11 1± 29
	do	38200	24	50 0± 67	22 3± 33	12 9± 17	13 1± 29
	do	39233	5	46 0±1 30	20 2± 37	10 5± 45	10 9± 29
Olson 68	do	38186	33	32 1± 57	17 5± 52	12 9± .21	11 8± 13
	do	39238	5	30 0±2 55.	14 0±1 95	10 9± 33	10 2± 11
34753 × tall type	F_1	37195	14	37 9±1 37	16 9± 27	12 6± 30	11 3± 34
	do	38203	5	47 4± 81	20 8± 58	14 3± 26	15 8± 20
(34753 × tall type) selfed	F_2	37196	70	39 2± 61	16 6± 35	13 3± 21	12 3± 19
(34753 × tall type) × 34753	do	37197	75	46 0± 86	17 7± 26	14 1± 24	12 7± 23
	do	38205	41	45 6±1 24	21 9± 16	14 6± 26	13 6± 29
(34753 × tall type) × tall type	do	37198	71	40 5± 57	18 6± 32	12 2± 21	11 8± 22
(34753 × tall type) × Olson 68	do	38222	80	51 9± 71	24 2± 47	14 9± 17	13 4± 16

The F_1 between strains 34753 and tall type was significantly taller than strain 34753, but was not as tall as the taller parent (table 1). It had significantly fewer leaves than either, and the largest leaf was at least as large as in the parents. Significance in this paper is determined by the t test for $P=0.05$. There was no decrease in average vigor from the first to the second hybrid generation. Backcrosses of the F_1 to strain 34753 (table 1) and to tall type tended to be intermediate between the F_1 and F_2 averages and those of the recurrent parent, with certain exceptions.

Some individuals with pronounced increases in size were obtained in the second hybrid generation. These were selfed and also were crossed with Olson 68 in an attempt to produce still larger plants. The immediate effect of Olson 68 on increasing plant height and number of leaves is evident by comparing (table 1) family 38205 with family 38222.

SELECTIONS FROM HYBRIDS

Four new lines were developed from the best plants in the second-generation families 37197 and 38222. They were continued by selfing and also were crossed once with Olson 68 and then successively selfed. Selections from the latter self-pollinations produced better plants than selections from the former. Average measurements on a few of the best families in each line, from the F_3 to the F_6, are shown in table 2. Data on some outstanding individuals are presented in table 3. The original F_2 plant of line 1 and its progeny were tall and had exceptionally large leaves, which was a major factor in producing the high yields of plant material obtained.

TABLE 2.—*Means of measurements on families in selected lines from hybrids between types of Nicotiana rustica*

Line No.	Generation	Family No.	Number of individuals	Average plant height	Average number of leaves	Average length of largest leaf	Average width of largest leaf
				Inches		*Inches*	*Inches*
	F_3	38208	69	48.2±0.98	21.2±0.45	15.5±0.19	14.2±0.20
Line 1	F_4	38224	72	51.2± .55	21.8± .25	16.3± .14	15.0± .15
	F_6	39293	5	52.2±2.58	25.2±1.59	13.1± .43	10.4± .43
	F_3	39245	8	46.3±3.03	23.8±1.84	11.9± .32	10.9± .52
Line 2	F_4	38228	42	44.6± .55	22.7± .58	15.1± .23	13.4± .20
	F	38278	19	50.4±1.52	27.5± .74	12.2± .22	9.6± .21
	F_4	38218	42	54.8± .83	26.0± .56	13.7± .13	12.0± .14
Line 3	F_4	38219	42	55.9± .64	27.9± .52	14.6± .18	12.4± .21
	F	39291	9	44.3±3.21	29.1± .83	11.3± .34	8.7± .28
Line 4	F	39271	10	41.7±1.27	22.9±1.07	11.7± .19	10.4± .43
	F_4	39292	6	58.7±1.48	25.0±1.16	11.7± .31	11.3± .34
Line 3 × line 1	F × F	39296	8	51.4±3.37	24.6± .87	12.9± .26	9.9± .26
Line 2 × line 1	$F_5 \times F_6$	39300	14	60.8±3.49	32.1±1.01	13.3± .26	10.7± .25

TABLE 3.—*Data on best plants in selections from hybrids*

Line and individual plant No.	Plant height	Number of leaves	Length of largest leaf	Width of largest leaf	Total weight of leaves	Nicotine, air-dry basis, plant not topped	Estimated potential yield of nicotine per acre if plants are topped
Line 1	*Inches*		*Inches*	*Inches*	*Grams*	*Percent*	*Pounds*
38208-62	48	26	19.0	15.5	135.9	4.55	153
Line 2							
39245-1	50	29	12.0	11.0	129.3	4.01	151
Line 3							
38219-20	60	29	16.0	14.5	163.0	4.45	204
Line 4							
38222-9	48	30	15.5	15.0	138.7	4.4	174
Line 3 × line 1							
39296-1	36	23	12.5	9.0	112.1	30	1[illegible]
Line 2 × line 1							
39300-4	53	34	14.0	11.5	145.0	6.22	231
39300-6	61	41	13.5	11.0	129.0	4.90	1[illegible]3
39300-9	51	31	14.5	11.0	127.0	4.19	20[illegible]

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[1] Topping is the practice of cutting off the upper part of the plant which includes the inflorescence and smallest leaves.

[2] Estimate based on a 2 percent increase in nicotine content if plants are topped. The yield from the stalks has not been added; this should amount to about 15 more pounds of nicotine per acre.

The original F_2 plant of line 2 and its progeny were large in all size characteristics measured and were late-flowering, a favorable feature. The original F_2 plant of line 3 and its progeny had relatively many leaves, which was a major factor in producing its high yields. Line 4 was developed from the best plant of family 38222, which was large and relatively high in nicotine content.

Many of the plants grown in 1939 were too stunted by the dry season to be considered typical; but when comparisons were made between families in which a limited number of representative individuals were measured, it was found that each new line was significantly larger than the best original types in one or more measurements. Increased size had been maintained, then, by selection and inbreeding through a number of generations and under adverse seasonal conditions.

In order to combine desirable characteristics of the new strains, crosses were made in all possible combinations among the best F_5 individuals in lines 1, 2, and 3 and the best F_3 in line 4. These F_1

crosses were each significantly larger (with one exception) than the original varieties. Especially promising was the cross between line 2 and line 1 (39300 in tables 2 and 3), which was superior to any of the new inbred lines, themselves. Line 3 × line 1 (39296) also proved to be a favorable combination.

In regard to the most outstanding segregants, plant No. 2 (fig. 2, *A*) of the cross line 2 × line 1 was 76 inches tall, which was the greatest height reached by any *Nicotiana rustica* grown during the course of the experiments. Plant No. 6 (fig. 2, *B*) of the same cross had 41 leaves, which was the highest leaf number recorded. Finally, the increased size of certain individuals was demonstrated by their high yield, which is discussed below.

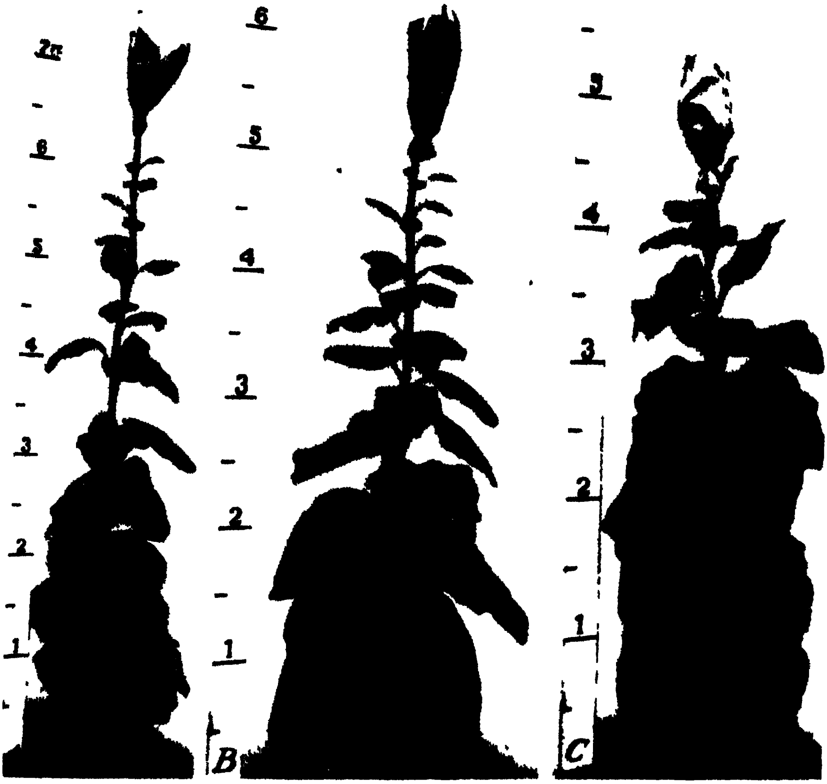

FIGURE 2. Three exceptional plants in the family (39300) produced by crossing line 2 × line 1: *A*, Plant No 2, height 76 inches. *B*, plant No. 6, 41 leaves. *C*, plant No 4, 145 gm. of leaf material, estimated potential yield of 246 pounds of nicotine per acre.

RESULTS ON YIELD OF NICOTINE

WEIGHT OF LEAF MATERIAL

Selection in these experiments was based on yield of nicotine as well as on characteristics of the plant in the field. The former is mainly a function of total leaf weight and percentage of nicotine. Green plants with the largest measurements in the field usually had the heaviest weight of dried leaf material, so that there was general correspondence between the two groups of data.

TABLE 4.—*Total weight of leaves, percentage of nicotine, and estimated potential yield of nicotine per acre from selected bagged plants of parental types of Nicotiana rustica*

Parental type	Family No	Number of individuals	Total weight of leaves		Nicotine, air-dry basis		Estimated potential yield of nicotine per acre	
			Average	Highest	Average	Highest	Average	Highest
			Grams	*Grams*	*Percent*	*Percent*	*Pounds*	*Pounds*
Variety *brasilia*, 34753	37190	10	94.8	115.8	2.71	3.19	50.3	71.7
	38179	7	88.0	119.9	4.25	5.84	75.1	94.2
Variety *brasilia* No 23	39231	4	41.2	63.9	6.15	7.24	47.9	68.4
Tall type	37192	9	74.5	91.2	2.09	2.73	31.5	48.3
	39233	4	44.2	50.5	3.44	4.11	28.7	34.1
Olson 68	38186	5	83.6	106.9	3.69	5.43	61.7	84.5
Olson 68A	39239	3	54.6	65.1	5.79	6.14	61.3	72.0

Weights for the parent strains are given in table 4, showing the average and maximum for each year. In 1939 data on strains 34753 and Olson 68 were not obtained, owing to an accident; so those on var. *brasilia* No. 23 and Olson 68A, two similar types, were substituted for comparison with hybrids grown that year.

TABLE 5.—*Total weight of leaves, percentage of nicotine, and estimated potential yield of nicotine per acre from F_1 and F_2 bagged plants of Nicotiana rustica hybrids*

Generation	Family No [1]	Number of individuals	Total weight of leaves		Nicotine, air-dry basis		Estimated potential yield of nicotine per acre	
			Average	Highest	Average	Highest	Average	Highest
			Grams	*Grams*	*Percent*	*Percent*	*Pounds*	*Pounds*
F_1	37195	2	87.7	119.1	2.51	3.68	49.8	85.0
	38203	4	91.9	106.2	2.27	3.46	40.0	59.9
F_2	37196	12	98.0	120.8	3.19	4.20	61.5	86.3
	37197	24	103.2	132.2	3.11	4.22	63.2	108.2
	38205	10	95.7	125.2	3.54	4.99	66.5	101.3
	37198	16	96.8	143.6	2.85	4.56	55.6	127.0
	38222	15	101.2	143.5	3.65	5.57	72.0	119.7

[1] See table 1 for designation of parents for each family

F_1's between strain 34753 and tall type (table 5) weighed about the same as strain 34753, the heavier parent. Best second-generation plants from the same cross were slightly heavier on an average than the F_1's and were outstanding in that the maximum weights were higher than in the parents or F_1's. This same result was obtained when germ plasm from Olson 68 was introduced (38222).

Plants in the new lines had the highest individual and average weights recorded during the experiments (tables 3 and 6). The dried leaf material alone, of the best plant in line 3, weighed 163 gm., which was the maximum weight obtained.

The best $F_5 \times F_5$ family, 39300, as judged by characteristics in the field, also had higher weights of leaf material than did any other family grown that year. Leaves of the heaviest plant, No. 4 (fig. 2, C), weighed 145 gm., which compared favorably with the weight of the plants grown during better seasons.

TABLE 6.—*Total weight of leaves, percentage of nicotine, and estimated potential yield of nicotine per acre in bagged plants of best families in the new lines of Nicotiana rustica*

Line No.	Family No	Number of individuals	Total weight of leaves		Nicotine, air-dry basis		Estimated potential yield of nicotine per acre	
			Average	Highest	Average	Highest	Average	Highest
			Grams	*Grams*	*Percent*	*Percent*	*Pounds*	*Pounds*
Line 1	38208	18	110.3	138.5	3.39	4.61	74.2	120.0
	39293	3	96.8	124.0	4.59	5.12	87.6	116.9
Line 2	39245	4	105.0	129.3	4.71	4.99	94.9	102.7
	39278	10	81.8	112.5	3.89	4.75	60.7	92.9
	38219	11	123.6	163.0	3.56	4.45	86.3	140.7
	39291	6	84.9	107.0	5.97	6.80	98.8	141.2
	39271	2	77.9	88.0	5.26	6.07	81.0	103.6
	39292	3	86.1	95.4	4.99	5.81	82.9	97.6
Line 3 × line 1	39296	4	95.3	112.4	4.10	5.30	76.9	115.6
Line 2 × line 1	39300	14	104.6	145.0	5.29	6.51	108.4	175.0

PERCENTAGE OF NICOTINE

Percentage of nicotine, like size of plant, is affected by the environment, so that pronounced yearly fluctuations occurred even in the homozygous parent strains.

Topping has a major influence on building up nicotine, and, from records over a 5-year period, it was found that topped plants had, on an average, an absolute increase of about 2.5 percent alkaloid as compared with bagged ones. An increase of 2 percent would be a conservative estimate of the effect of topping; this value corresponds closely to results obtained in New York State for 1924 and 1925.[4] Since all determinations in tables 4, 5, and 6 were made on bagged plants, approximately 2 percent should be added in order to estimate their potential content.

There was no marked gain or loss in percentage of nicotine from the higher parent to selected plants of the F_2 generation (table 5). Addition of Olson 68 in crosses caused an increase in nicotine production in both the F_2 and later generations.

The new lines were somewhat lower in percentage of nicotine than the parental types 34753 and Olson 68, but owing to their larger size some plants in most families were higher in yield of nicotine than the parents (table 6).

YIELD OF NICOTINE

Yield of nicotine per plant is the product of the weight and the percentage of nicotine. It was considered advisable to multiply this figure by the number of plants per acre, which would not change the relative individual values, and yet would provide some basis for estimating whether or not the increases were sufficiently great to be of possible economic importance.

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[4] COLLISON, R. C., HARLAN, J. D., and STREETER, L. R. HIGH-NICOTINE TOBACCO. N. Y. State Agr. Expt. Sta. Bul 562, 19 pp. 1929.

For bagged plants of the best parent strain during the years of the experiment an estimated maximum yield of about 94 pounds of nicotine per acre and an average of 75 were obtained (38179 in table 4).

The new lines contained bagged individuals with estimated yields as high as 141 pounds, and the average in a number of families was higher than in the parents (table 6). Fourteen selected bagged plants of the best $F_5 \times F_5$ family had an estimated maximum potential yield of 175 pounds of nicotine and an average of 108 pounds, obviously significant increases over the original varieties.

When the effect of topping and the yield from stalks are included, the potential values are still higher (see table 3, last column).

DISCUSSION

GENETIC CONSIDERATIONS

Increased height in intervarietal hybrids of *Nicotiana rustica* was reported by East [5] in crosses between the varieties *brasilia*, *texana*, *scabra*, and *humilis*. From the data obtained he concluded that reciprocal F_1's were very similar to each other and that the parent varieties differed by many genes for size. Hybrid vigor in first-generation plants from crosses between certain strains of *Nicotiana rustica*, but not others, was reported by Bolsunov [6] in Russia.

The F_1 described in these experiments was not larger than the parents, though other *Nicotiana rustica* crosses, less promising for nicotine production, did show typical hybrid vigor. The data reported present difficulties for genetic analysis, but certain general conclusions regarding the genetic basis for the results obtained appear tenable.

In crosses between strain 34753 and tall type it was found that the F_1 was almost as tall as the taller parent; the F_2 contained some plants that were taller than the parents or F_1; and in the F_4, families with significantly different mean heights were produced, one of which was significantly taller than tall type. These results, combined with observations on the backcrosses, were interpreted to mean that the parents differed by partially dominant genes for increasing height, most of which were from tall type. Some were contributed by strain 34753; so by genic recombination segregants and subsequent families were produced that exceeded the taller parent in height.

In crosses between strain 34753 and tall type it was found that the F_1 had fewer leaves than either parent. The F_2 generation did not differ significantly from the F_1 in average number of leaves and contained segregants that were equal, but no better, in leaf number than the original types. Backcrosses of the F_1 to each parent had significantly higher means for leaf number than the F_2. F_4 families with significantly different means occurred. Two F_4's and one F_6 had a significantly higher average number of leaves than the better parent. It was concluded that increase in leaf number in these selections was controlled by genes, recessive in their action, some of which were contributed by each parent.

Tall type and strain 34753 do not differ significantly in length or width of the largest leaf. The F_1 was significantly larger according to 1938 results. The F_2 and first backcross generation showed a slightly increased variability over the F_1. Leaves of the backcross to strain 34753 were especially large. F_4 families were obtained that had significantly larger leaves than either parent. It was concluded that, although strain 34753 and tall type had approximately the same size largest leaf, the genes controlling this characteristic differed in the two types.

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[5] EAST, E M A STUDY OF PARTIAL STERILITY IN CERTAIN HYBRIDS Genetics 6 [311]-365, illus 1921

[6] BOLSUNOV, I I. CONTRIBUTION TO THE STUDY OF HYBRID VIGOUR IN NICOTIANA RUSTICA L III DYNAMICS OF HYBRID GROWTH Vsesoiuzn Nauch. Issled. Inst. Tabach. i Makhor. Promysh [Krasnodar] [Bul] 139 [44]-57, illus. 1939 [In Russian. English summary, pp. 56-57.]

There was evidence that the genes controlling the inheritance of plant height, number of leaves and size of the largest leaf were to some extent independent, an important factor in recombining desirable traits into new strains. The genotype of Olson 68 apparently contains more genes for increasing plant height than strain 34753, it can add to the number of leaves in certain genic combinations, and in no selections studied did the addition of Olson 68 cause a significant difference in leaf size.

In table 7, the frequency distributions of measurements on plant height, number of leaves, and length of the largest leaf are arranged for 34753, tall type, F_1, $F_1 \times 34753$, best F_4, and best $F_5 \times F$. The families involve only strain 34753 and tall type, with the exception of the best $F_5 \times F$, which contains germ plasm from Olson 68 as well as from the other two parents. Table 7 shows the variability in the families concerned and gives some idea of the effect of hybridization and of selection on the characteristics measured.

TABLE 7.—*Frequency distributions of plant height, number of leaves, and length of largest leaf for two parent types and selected hybrid families*

PLANT HEIGHT

Parent or hybrid	Frequency distribution of plant height in inches																											Mean height
	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60	62	64	66	68	70	72	74	76	
34753	4	2	6	8	7	3																						29.9
Tall type										1	1	4	3	5	8	1	1											50.0
F_1											1	1	3															47.4
$F_1 \times 34753$				3	1	2	1	3	3	7	3		2	4	0	3	4	2	0	1								45.6
Best F_4									1	1	0	2	4	4	3	3	6	9	8	1								54.8
Best $F_5 \times F$				1	0	0	0	0	0	1	0	0	0	1	1	0	0	0	3	1	0	0	2	0	3	0	1	60.8

NUMBER OF LEAVES

Parent or hybrid	Frequency distribution of number of leaves															Mean number of leaves
	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	
34753	1	2	0	0	2	5	6	6	6	2						24.5
Tall type				1	6	10	7									22.3
F_1				1	2	2										20.8
$F_1 \times 34753$		1	6	2	10	8	12	4	1							21.9
Best F_4			1	1	1	6	4	10	13	5	6	1				28.0
Best $F_5 \times F$								1	3	3	1	5	0	0	1	32.1

LENGTH OF LARGEST LEAF

Parent or hybrid	Frequency distribution of leaf length in inches											Mean length
	10	11	12	13	14	15	16	17	18	19	20	
34753		2	5	15	8							13.1
Tall type		3	7	11	3							12.9
F_1				1	3	1						14.3
$F_1 \times 34753$	2	2	2	4	9	4	9	1	1			14.6
Best F_4	1	1	2	2	7		12	3	4	0	1	15.3

There were insufficient data to warrant drawing any conclusions regarding the genetic factors controlling nicotine content. It was evident, however, that either some genes for nicotine production from strain 34753 and Olson 68 were not included in the genotypes selected for the new lines, or else larger plants are not capable of building up as high a percentage of nicotine as smaller ones under the same conditions.

ECONOMIC CONSIDERATIONS

On the basis of prices paid for tobacco sold for byproduct, the returns per acre for crops containing 100 to 150 pounds of nicotine would compare favorably with the average returns over a period of years from culture of at least some types of commercial leaf tobacco. Topped plants of the original varieties actually gave such yields under the conditions described here, and the production of alkaloid may be increased somewhat by allowing the plants to remain longer in the field from time of topping to harvest. The costs of culture and of nicotine extraction are not definitely known, however, and it has been estimated that the profit, if any, from growing the original varieties for nicotine would be small.

The results on best selections in the new lines, to the F_6 generation, indicate that it may be possible to grow types of *Nicotiana rustica* that yield up to 200 pounds of nicotine per acre under the cultural and environmental conditions of these experiments. If the homozygous strains now being developed are able to produce as high yields in field tests, it should be possible to grow *N. rustica* profitably for the nicotine alone.

The cost of preparing *Nicotiana rustica* for market would be less than for commercial leaf tobacco; but, since it forms suckers more abundantly than *N. tabacum*, more labor would be required for their removal. In this connection, Bolsunov[7] has recently described an unusual variant, resulting from a cross between two types of *N. rustica*, in which the axillary buds were either partially atrophied or completely absent and in some cases of which the terminal inflorescence was also lacking. Seed was obtained from secondary suckers on the lower part of the plants, and selection to the F_5 generation has produced lines which still show the anomaly. This type, if it is as promising as described, should be of value, since the cost of topping and suckering would be reduced.

SUMMARY

New lines of *Nicotiana rustica*, developed by hybridization and selection, were significantly larger on an average than their three parental strains, namely, *N. rustica* var. *brasilia* (strain 34753), tall type, and Olson 68.

The characteristics measured were plant height, number of leaves, and size of largest leaf. These were controlled by relatively independent genes that could be recombined in advantageous groupings. The best parent values were usually not exceeded until new homozygous combinations were established in the F_2 or later generations. The original strains differed by some genes for each character.

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[7] BOLSUNOV, I. I. [A VALUABLE HYBRID OF NICOTIANA RUSTICA DEVOID OF INFLORESCENCE AND UPPER SUCKERS.] Selek. i Semen. 2-3 40-41, illus. 1939. [In Russian.]

Further increases in size were obtained by crossing F_5 selections of the new lines together. The occurrence of some exceptionally large segregants suggests that still further increases in the average size will be possible.

Related data on the weight of dried leaf material and percentage of nicotine were reported. Estimated potential yields of nicotine per acre were calculated from these figures, and selections in the new strains, to the F_6 generation, were found to be superior to their original parents in this respect. The possible economic importance of the improved lines, for use as a source of nicotine for insecticidal purposes, was discussed.

SOME FACTORS THAT INFLUENCE THE IMMEDIATE EFFECTS OF POLLEN ON BOLL CHARACTERS IN COTTON[1]

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INTRODUCTION

In 1931 Harrison (*2*)[3] reported the occurrence of immediate effects of pollen on six boll characters of cotton. As Swingle (*7*) had defined metaxenia as the immediate effect of pollen on the parts of the seed and fruit lying outside the embryo and endosperm, Harrison considered that only the characters of boll period, lint length, and seed fuzziness exhibited metaxenia. The design of the experiment performed by Harrison in 1929 was followed by the writer in experiments performed at the United States Field Station, Sacaton, Ariz., in 1934, 1935, and 1936, except that inbred strains of cotton were used exclusively. In these later experiments the effects of three kinds of pollen were compared: Pima Egyptian from a strain inbred 20 years, Acala upland from a strain inbred 11 years, and Hopi from a strain inbred 12 years, these pollens having been used to fertilize emasculated flowers of Acala of the same strain as that which furnished the Acala pollen.

Differences of smaller magnitude than those reported by Harrison, but usually significant, were found in respect to each of the five characters determined in these later experiments. Kearney (*3*) in 1929 and Harrison (*2*) in 1931 reported that boll-maturation period was affected by the amount of exposure to sunlight. The existence of other factors that can mask or emphasize the expression of the immediate effects of pollen on cotton was demonstrated by the experiments reported in this paper. The factors referred to are the effects of (1) variation in seasonal conditions from year to year, (2) variations in weather or other conditions on or near the day of anthesis, (3) individual variation among the plants used as pistillate parents, and (4) variation in conditions affecting the nutrition of the bolls.

MATERIALS AND METHODS

It was sought to determine the effects of pollen in bolls resulting from two interspecific cross-pollinations, A × P (*Gossypium hirsutum* L. × *G. barbadense* L., Acala × Pima) and A × H (*G. hirsutum* × *G. hopi* Lewton, Acala × Hopi), and one endogamic cross-pollination, A × A (*G. hirsutum* × *G. hirsutum*, Acala × Acala) in respect to five characters of the bolls, namely, number of seeds, seed index,[4] lint index,[5] fiber length, and boll-maturation period.

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[1] Received for publication March 31, 1941.

[2] The author is indebted to E. Gordon Smith, assistant scientific aide, Division of Cotton and Other Fiber Crops and Diseases, for generous assistance in the field and in the laboratory.

[3] Italic numbers in parentheses refer to Literature Cited, p. 480.

[4] Seed index is the equivalent of the weight, in grams, of 100 seeds.

[5] Lint index is the equivalent of the weight, in grams, of the lint removed from 100 seeds.

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Fifteen Acala plants were selected as pistillate plants each year, and each plant received the 3 cross-pollinations in rotation as flowers appeared throughout the pollinating season. Fifty undamaged bolls of each cross-pollination were obtained in 1934 and in 1936, but the 1935 material was discarded, except for fiber-length determinations from undamaged locks, because of the very small number of normal bolls obtained before frost. In 1934 the 150 bolls were obtained from pollinations made on the 10 days (Saturday and Sunday excepted) between August 15 and August 27. In 1936 they were obtained from pollinations made on 18 days between August 1 and August 27.

INTERRELATIONSHIPS OF BOLL CHARACTERS STUDIED

Table 1 gives the set of coefficients of correlation for the 5 boll characters considered in this study. The "within seasons" value of *r* is used in order to have a figure that would most nearly correspond to one that could be expected if all 300 bolls had been grown in one year. Number of seeds per boll is negatively correlated with seed index, with lint index, and with fiber length. The fact that fewer seeds per boll resulted in heavier seeds with more abundant and longer lint indicates that a nutritional factor is involved. Assuming that a definite quantity of plant food is furnished each boll, it is a logical assumption that a few seeds utilizing this food would grow larger and produce more and longer lint than if the same amount of food were utilized by a larger number of seeds.

TABLE 1.—*"Within seasons" value of the correlation coefficients of characters determined on 300 bolls of cotton grown on Acala plants in 1934 and in 1936 as a result of cross-pollinations A × P, A × A, and A × H*

Character and population	Correlation with			
	Seed index	Lint index	Fiber length	Boll period
Seeds per boll				
As one array	−0.6899	−0.3496	−0.3223	−0.0001
A × P	−.6490	−.2424	−.3055	+.0680
A × A	−.8290	−.4315	−.3554	+.1175
A × H	−.6764	−.2646	−.3377	−.0563
Seed index				
As one array		+.3741	+.5491	−.1548
A × P		+.5356	+.7117	−.2771
A × A		−.0088	+.3008	−.2200
A × H		+.3445	+.5210	−.0820
Lint index				
As one array			+.1834	+.1067
A × P			+.4243	+.0374
A × A			+.0704	+.1173
A × H			−.0257	+.1195
Fiber length				
As one array				−.1745
A × P				−.2397
A × A				−.1348
A × H				−.1670

[1] In "As one array," where $N=300$, r should be ±0.148 at $P=0.01$, in the individual cross-pollinations, where $N=100$, r should be ±0.254 at $P=0.01$ (8), N=number of observations.

The correlation coefficients of seed index with lint index show that, with the exception of cross-pollination A × A, heavy seeds are associated with abundant fiber. All three cross-pollinations produced longer fiber on heavier seeds than on light seeds. The negative correlation of seed index with boll period in cross-pollination A × P and A × A shows that as a rule heavier seeds mature faster than lighter ones. The lack of correlation of seed index with lint index in cross-pollination A × A and the existence of a correlation of seed index with boll period when there was no correlation of number of seeds per boll with boll period indicate that heterosis is also involved. The heavy seeds in cross-pollinations A × P and A × H are apparently larger and have a more extensive surface from which to develop fibers. The lack of correlation of seed index with boll period in the material from cross-pollination A × H, although such a correlation resulted in cross-pollinations A × P and A × A, demonstrates the difficulty of segregating the effects of heterosis from those of nutrition.

As Pope (*4, pp. 744 747*) has shown a relatively uniform weight per unit length of fiber in the Acala group, the increased weight of fibers on the heavy seeds might be due in part to the increased length. However, only cross-pollination A × P showed a significant positive correlation of lint index with fiber length.

In the three populations as one array, the negative correlation of fiber length with boll period shows that the factors that promote the development of long fibers tend to hasten maturity, adding another indication that nutrition is a factor involved in these results.

The use of inbred strains of cotton to eliminate significant variation among plant means was only partly successful. Table 2 shows that for each of the five characters the differences between means of the pistillate plants receiving all cross-pollinations ranged from zero to a maximum greater than the maximum difference between the means of the populations receiving the several cross-pollinations. A record of the staminate parents was made in 1936. There was no significant difference in number of seeds per boll among means of staminate plants furnishing pollen for any of the three cross-pollinations. In view of the highly significant correlation of this character with seed index, with lint index, and with fiber length, it was assumed that a uniform supply of pollen had been used for each cross-pollination. The small number of staminate plants involved, five of Pima, four of Acala, and two of Hopi, undoubtedly reduced the opportunity for wide variation among their means.

TABLE 2.—*Range of differences and average of differences among means of cross-pollinations, between means of seasons, among means of day of anthesis within seasons, and among means of pistillate plants within seasons for each of the 5 characters determined on 300 bolls of cotton produced by Acala plants in 1934 and in 1936 as a result of pollination with Pima, Acala, and Hopi pollens*

Means compared	Number of comparisons	Character									
		Seeds per boll		Seed index		Lint index		Upper quartile fiber length		Boll period	
		Range	Average	Range	Average	Range	Average	Range	Average	Range	Average
Cross pollination	3	2 53-3 77	3 85	0 11-1 06	0 71	0 05 0 34	0 23	0 003-0 016	0 011	0 23-0 95	0 63
Seasons	1		2 16		77		24		046		3 23
Day of anthesis											
1934	45	0 11 5	4 69	01 2 90	1 06	0 1 17	37	0 - 1.30	052	10-6 70	2 65
1936	153	0 7 7	2 21	01 2 14	60	0 1 39	45	0 - 080	023	0 16 0	6 36
Pistillate plants											
1934	105	1 - 7 1	2 55	0 -2 2	72	0 1 2	32	0 - 055	021	0 - 5 9	1 70
1936	105	0 -10 8	2 47	0 -1 1	43	0 9	30	0 - 038	013	0 12 5	3 43

EFFECTS OF CROSS-POLLINATIONS

In the comparison of means of cross-pollinations shown in table 3, the differences between cross-pollinations A × P and A × A are significant in respect to all five characters. The use of Pima pollen on Acala plants, in comparison with the use of Acala pollen on the same plants, reduced the number of seeds per boll, increased the weight of the seeds, increased the amount of lint on the seeds, lengthened the fibers, and required a longer time to mature the bolls.

Hopi pollen, as shown in the comparison of cross-pollinations A × H and A × A, increased significantly the number of seeds per boll and the fiber length. The differences between means of seed index, lint index, and boll period were not significant. The increased fiber length in cross-pollination A × H was the reverse of the results obtained by Harrison (*2*). As he used the same strain of Hopi as that used in the experiments reported in this paper, the consistent increase in length obtained in 1934, 1935, and 1936 was unexpected. This apparent reversal of the effects of Hopi pollen on fiber length will be discussed further in the section on effects of specific pistillate plants.

When cross-pollinations A × P and A × H are compared, significant differences between means of all characters except fiber length are shown. Cross-pollination A × P yielded fewer seeds per boll than cross-pollination A × H and gave larger means for seed index, lint index, and boll period

TABLE 3.—*Comparison of the means* [1] *of cross-pollinations for each of the five characters determined on 100 bolls of cotton of each of the three cross-pollinations, A × P, A × A, and A × H, grown in 1934 and 1936*

Cross-pollination	Character									
	Seeds per boll (number)		Seed index (grams)		Lint index (grams)		Upper quartile fiber length (inches)		Boll period (days)	
	M	t	M	t	M	t	M	t	M	t
A × P A × A	25.57 28.81		12.79 11.84		6.70 6.36		1.0288 1.0129		58.25 57.53	
Difference	−3.24±0.373	8.69	+.95±0.063	15.08	+.34±0.045	7.55	+0.159±0.0034	4.68	+.72±0.267	2.70
A × H A × A	31.34 28.81		11.73 11.54		6.41 6.36		1.0262 1.0129		57.30 57.53	
Difference	+2.53±.307	6.89	−.11±.062	1.77	+.05±.044	1.14	+.0133±.0033	4.03	−.23±.262	.88
A × P A × H	25.57 31.34		12.79 11.73		6.70 6.41		1.0288 1.0262		58.25 57.30	
Difference	−5.77±.365	15.81	+1.06±[illegible]	17.10	+.29±.044	6.59	+.0026±.0033	.79	+.95±.261	3.64

[1] See Wallace and Snedecor (8, pp. 62-63) for a table of t values.

FACTORS THAT MODIFY THE IMMEDIATE EFFECTS OF POLLEN

VARIATIONS IN SEASONAL CONDITIONS FROM YEAR TO YEAR

Table 2 shows that the difference between the means of seasons in number of seeds per boll is smaller than the minimum difference between means of cross-pollinations. In seed index and in lint index the difference between means of seasons approximates the average of the three differences among means of cross-pollinations. In fiber length and in boll period the difference between means of seasons is approximately three times the maximum difference between means of cross-pollinations.

The influence of season in modifying or changing the effects of different pollens is best illustrated in seed index, where cross-pollination and season interaction exists (see table 4). The difference of 0.77 gm. between means of seasons shown in table 2 represents an increase in 1936 equal to 6.7 percent of the 1934 mean of all cross-pollinations as one array. The increase in cross-pollination A × P was 3.8 percent of its 1934 mean, that in cross-pollination A × A was 10.4 percent of its 1934 mean, and that in cross-pollination A × H was 6.2 percent of its 1934 mean. By extending these experiments over two seasons, the magnitude of the differences among means of cross-pollinations in seed index is smaller than would have been the case if only the results of 1934 had been used.

TABLE 4.—*Level of significance of differences among means of factors affecting boll characters of cotton as shown by analyses of variance of 100 bolls each of 3 cross-pollinations grown in 1934 and in 1936 (group A), and of 15 bolls of each of 3 cross-pollinations, 3 bolls of each cross-pollination with 29, with 30, with 31, with 32, and with 35 seeds per boll, grown in 1936 (group B)*

Items	Differences among means of factors affecting character indicated				
	Number of seeds	Seed index	Lint index	Upper quartile fiber length	Boll period
Group A					
Cross-pollinations	0.01	<0.01	0.01	>0.05	0.05
Seasons	<.01	<.01	.01	>.05	<.01
Day of anthesis	<.01	<.01	.01	<.01	<.01
Pistillate plants	.05	0.05-.01	0.05-.01	.05	>.05
Bolls with many and few seeds	<.01	<.01	.01	<.01	.05
Interactions					
Cross-pollinations—seasons	.05	<.01	>.05	>.05	>.05
Cross-pollinations—day of anthesis	<.01	<.01	>.05	>.05	>.05
Cross-pollinations—pistillate plants	>.05	<.01	>.05	.05-.01	.05-.01
Cross-pollinations—many and few seeds	<.01	.05	.05	.05	>.05
Seasons—many and few seeds	.01	<.01	>.05	<.01	>.05
Cross-pollinations—seasons—many and few seeds	<.01	<.01	>.05		>.05
Group B					
Cross-pollinations		>.05	>.05	>.05	>.05
Seeds per boll		>.05	.05	>.05	>.05
Interaction					
Cross-pollinations—seeds per boll		>.05	>.05	>.05	.05

VARIATIONS IN WEATHER OR OTHER CONDITIONS ON OR NEAR THE DAY OF ANTHESIS

The average and maximum differences among means of day of anthesis, shown in table 2, seem large when the short periods of time, during which pollinations were made are considered. In all five characters the maximum difference between means of day of anthesis in each season was larger than the maximum difference between means of cross-pollinations. Some weather phenomena of the day of anthesis, or of the days preceding or following it, are presumed to have contributed to these effects; or there may have been a cumulative effect of some phenomena operating over a period of several days preceding, or possibly following, the day of anthesis. Unsuccessful attempts were made to correlate the number of seeds per boll with maximum, with minimum, and with average temperatures, and with the average relative humidity percentage of the day of anthesis. There was a slight indication that number of seeds per boll and evaporation from an open tank 5 days after anthesis might be negatively correlated, but the value of r was far from significant. A study of the average yields of American-Egyptian cotton in Arizona showed a negative correlation of yields with July and August evaporation from an open tank at Sacaton (*1*).

The importance of the effects of day of anthesis on number of seeds per boll in this study can be illustrated by examples of cross-pollination and day of anthesis interaction, or the differential response of the three pollens to the conditions of the day, or of days near the day, on which the crosses were made. On days 4 and 5 in 1934 (August 18 and 21) the mean numbers of seeds per boll were 23.0 and 29.6 respectively, a difference of 6.6 seeds. This difference of 6.6 seeds per boll was due to the fact that cross-pollination A × P yielded on the average 13.2 more seeds per boll on day 5 than on day 4; cross-pollination A × A responded with an average of 11.6 more seeds per boll; but cross-pollination A × H gave an increase of only 1.6 seeds per boll. Thus, although the conditions on day 5, in comparison with day 4, were advantageous to all three kinds of pollen, cross-pollinations A × P and A × A responded to a much greater extent than cross-pollination A × H. If conditions similar to those of day 5 had existed throughout the entire pollination period, the means for the several cross-pollinations at the end of the season would have been greatly changed. In 1936 the mean numbers of seeds per boll on days 14 and 15 (August 22 and 23) were 32.2 and 28.2 respectively, an average difference of 4.0 seeds per boll. Cross-pollination A × P gave an average of 5.4 fewer seeds on day 15 than on day 14, and cross-pollination A × A showed an average decrease of 7.7 seeds per boll; but cross-pollination A × H averaged 1.0 seed more per boll on day 15 than on day 14. If the conditions that existed on day 15 in 1936 had predominated throughout the entire pollination period, cross-pollinations A × H would have had a decided advantage over cross-pollinations A × P and A × A in number of seeds per boll.

The effect of day of anthesis on seed index showed day 5 in 1934 with a decrease in seed index from day 4 of 0.43 gm. for cross-pollination A × A, of 1.57 gm. for cross-pollination A × H, and of 3.58 gm. for cross-pollination A × P. In 1936 day 15 showed an increase in seed index over day 14, cross-pollination A × H increasing only 0.10 gm., and cross-pollination A × P only 0.15 gm., but the mean seed index of cross-pollination A × A was increased by 1.37 gm. Cross-pollination and day of anthesis interaction is not significant in the three remaining characters.

VARIATION AMONG PLANTS USED AS PISTILLATE PARENTS

Differences among the means of pistillate plants were above the 5 percent level of significance in fiber length and in boll period, and on the 5-percent or between the 5-percent and 1-percent level in number of seeds per boll, in seed index, and in lint index (table 4). However, the differences in fiber length among the means of pistillate plants provides an explanation of the unexpected lengthening effects of cross-pollination A × H in this character and will be used to illustrate these effects.

Two plants of each season were selected at random for this purpose. These are plants 5 and 9 of 1934 and plants 7 and 8 of 1936. In 1934 the average upper quartile fiber lengths of plants 5 and 9 were 0.98 and 1.00 inch respectively, plant 9 having bolls with fiber 0.02 inch longer than plant 5. Cross-pollinations A × A and A × H both gave fibers averaging 0.01 inch longer on plant 9 than on plant 5, while fibers of cross-pollination A × P were 0.06 inch longer on plant 9. In 1936 there was no difference between the means of plants 7 and 8 for bolls of cross-pollinations A × A and A × H, the small difference of cross-pollination A × P being only 0.002 inch in favor of plant 7.

An examination of plant means of the 1934 material showed that 1 plant did not produce bolls of both cross-pollinations A × A and A × H, and of the remaining 14 plants, 5 had means of cross-pollination A × H smaller than those of cross-pollination A × A In 1936, 2 of the pistillate plants did not have bolls of both of these cross-pollinations, 1 plant had the same mean for both, 6 plants had means of cross-pollination A × A exceeding those of cross-pollination A × H, and there were 6 plants in which cross-pollination A × H exceeded cross-pollination A × A in fiber length. The differences on the 6 plants where the fibers of cross-pollination A × H were longer than those of cross-pollination A × A were large enough to mask entirely the effects shown on the other 6 plants where the differences were reversed.

Designating Harrison's (*2*) cross-pollination Durango × Durango as D × D, and his cross-pollination Durango × Hopi as D × H where he used 25 Durango plants as pistillates in 1929, an examination of his original records shows that 13 of the 25 plants gave means for fiber length that were higher in D × H than in D × D. These include 50 of the D × H bolls, with a mean of 4.90 grades, and 52 of the D × D bolls, with a mean of 4.78 grades. The difference of 0.12 grade is equivalent to a lengthening effect of Hopi pollen of 0.015 inch. On the other 12 pistillate plants the 50 D × H bolls had a mean fiber length of 4.36 grades and the 48 D × D bolls a mean of 4.84 grades. The difference of 0.48 grade, or 0.06 inch, was sufficiently large to mask the lengthening effect of the Hopi pollen exhibited by the other 13 plants.

Pressley (*6, pp. 272–273*) also demonstrated an influence of individual differences in pistillate plants upon the effects of different pollens, his unit of comparison being a pair of seeds from near the center of a lock, one selfed the other a hybrid.[6] Thus his selfed and hybrid seeds had a maximum of uniform conditions for growth and development In his comparison of Pima selfed with Pima-Red Acala hybrids, the mean fiber length of the selfed seeds on plant 7 was 9/128 inch longer than those on plant 2, while the hybrid seeds of these pairs averaged 16/128 inch longer on plant 7 than on plant 2.

These examples of the effects of differences in individual pistillate plants as to fiber length show that effects of the pollen used may be modified or completely reversed by the selection of the pistillate plants used in the experiment.

VARIATION IN CONDITIONS AFFECTING NUTRITION

The correlations existing in the material used in these experiments, especially those of number of seeds per boll with seed index, with lint index, and with fiber length, indicate that nutrition was a factor affecting the expression of these characters. In 1929 Kearney (*3*) reported that the removal of the involucre of Pima cotton at the time of anthesis caused a marked reduction in the size and weight of the boll, in the weight of seeds, and in the abundance of lint. When the involucre was removed about 12 days before anthesis, the same was true and there was also a reduction in the number of seeds in the boll, presumably due to the undernutrition and resulting sterility of some of the ovules. In order to obtain the significant differences that Kearney found, it must be assumed that the Pima cotton plant furnishes food to the individual boll within a limited range; otherwise the effect of removing the involucre from the bolls would have been masked by the bolls receiving additional food from the unlimited central supply of the plant.

In table 5 the 1934 and 1936 material has been regrouped into classes of bolls with "many seeds" and with "few seeds." Table 6 gives the range of the number of seeds in each of these two classes. When the material from the three cross-pollinations is taken as one array, highly significant differences are shown between the means of bolls with relatively few and relatively many seeds in the characters, seed index, lint index, and fiber length. In cross-pollinations A × P and A × A significant differences between means for bolls with few and with many seeds are shown only in seed index and in fiber length. In cross-pollination A × H the only significant difference was in seed index. These data clearly show that a reduction in the number of seeds per boll results in heavier seeds that have longer and possibly more abundant lint. Porter (*5*), working with 10 varieties or strains of upland cotton grown at Greenville, Tex., in the years 1931, 1932, and 1933, found that number of seeds per lock was negatively correlated with fiber length. This is another illustration of a reduction in number of seeds resulting in longer lint.

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[6] Pressley applied pollen of 2 varieties of cotton to the stigmas of his pistillate plants From the resulting bolls he worked up data on a large number of pairs of seeds, each pair from near the center of an individual lock. The next season these seeds were grown to determine their male parentage, and only data from those pairs that consisted of 1 self-fertilized seed and 1 cross-pollinated seed were used in reporting his experiment

TABLE 5.—*Comparison of the means*[1] *of bolls with "few" seeds and of bolls with "many" seeds for each of the 5 characters determined on the 100 bolls of each cross-pollination grown on Acala plants in 1934 and 1936*

Means compared	Bolls	Seeds per boll	Seed index	Lint index	Upper quartile fiber length	Boll period
All cross-pollinations combined	*Number*	*Number*	*Grams*	*Grams*	*Inches*	*Days*
Bolls with few seeds	154	24.5	12.66	6.59	1.035	57.4
Bolls with many seeds	146	32.8	11.54	6.39	1.009	58.0
Difference		−8.3±.41	1.12±.125	.20±.071	.026±.0057	−.6±0
t		20.24	8.96	2.82	4.56	1.00
Cross-pollination A × P						
Bolls with few seeds	54	21.1	13.46	6.79	1.041	57.5
Bolls with many seeds	46	30.8	11.96	6.59	1.013	59.1
Difference		−9.7±.72	1.50±.217	.20±.123	.028±.0098	−1.6±1
t		13.47	6.91	1.63	2.86	1.54
Cross-pollination A × A						
Bolls with few seeds	47	24.4	12.39	6.42	1.028	57.0
Bolls with many seeds	53	32.7	11.35	6.31	1.000	58.1
Difference		−8.3±.72	1.04±.217	.11±.123	.028±.0098	−1.1±1
t		11.53	4.79	.89	2.86	1.06
Cross-pollination A × H						
Bolls with few seeds	53	28.1	12.08	6.52	1.035	57.7
Bolls with many seeds	47	34.9	11.34	6.28	1.015	56.9
Difference		−6.8±.72	.74±.217	.24±.123	.020±.0098	.8±1
t		9.44	3.41	1.95	20.4	.77

[1] See Wallace and Snedecor (*8, pp* 62–63) for a table of *t* values

TABLE 6.—*Range in number of seeds per boll in "many-few" classification given in table 5*

Cross pollination	Bolls produced in 1934 with—		Bolls produced in 1936 with—	
	Few seeds	Many seeds	Few seeds	Many seeds
A × P	9–23	24–36	13–28	29–42
A × A	14–28	29–40	16–30	31–36
A × H	4–31	32–42	16–32	33–38

If the immediate effect of different pollens were determined by nutrition alone, and if each cross-pollination were represented by an equal number of bolls, all having the same number of seeds per boll, there would be no differences among means of cross-pollinations. In the 1934 material the frequency distribution of number of seeds per boll was so irregular that 2 was the maximum number of bolls that would equally represent all three cross-pollinations, and that was available in only the class of 29 seeds per boll. In the 1936 material it was possible to select 3 bolls of each cross-pollination in each of 5 classes, those of 29, 30, 31, 32, and 35 seeds per boll. The means of these 15 bolls from each cross-pollination are compared in table 7. There are no significant differences among means of cross-pollinations in any of the 4 characters, although the difference between the mean lint index of cross-pollinations A × H and A × A approaches significance. Although the populations are small in the comparisons presented in table 7, the effects of seasons and of number of seeds per boll have been eliminated by selection of material. If it were possible to eliminate also the effects of day of anthesis and of variations among individual pistillate plants, the importance of nutrition in modifying effects of different pollens might be more clearly seen.

TABLE 7.—*Comparison of means*[1] *of 15 bolls of each cross-pollination, 3 bolls of each with 29, with 30, with 31, with 32, and with 35 seeds per boll, for the 4 remaining characters determined on bolls grown in 1936*

Cross-pollination	Seed index	Lint index	Upper quartile fiber length	Boll period
	Grams	*Grams*	*Inches*	*Days*
A × P	12 51	6 71	1 039	59 5
A × A	12 29	6 30	1 033	59 5
Difference	22± 267 82	41± 228 1 80	006± 0133 45	0±2 25
A × H	12 23	6 77	1 040	58 6
A × A	12 29	6 30	1 033	59 5
Difference	− 06± 267 22	47± 228 2 06	007± 0133 53	− 9±2 25 40
A × P	12 51	6 71	1 039	59 5
A × H	12 23	6 77	1 040	58 6
Difference	28± 267 1 05	− 06± 228 26	− 001± 0133 01	9±2 25 40

[1] See Wallace and Snedecor (8, *pp 62 69*) for a table of *t* values

The differences between the means of cross-pollinations A × P and A × A, and a study of the correlations existing among the characters in each of these cross-pollinations, indicate that nutrition, as determined by a differential in ability of the two pollens to effect fertilization of the ovules, is a primary factor in determining the immediate effect of pollen. There is a possibility that physical crowding, instead of nutrition, may be the primary effect of number of seeds per boll. However, the correlations existing in cross-pollination A × H and a comparison of the differences between the means of A × H and A × A with the differences between the means of A × P and A × A indicate that nutrition exerts a greater influence than physical crowding. When the means of cross-pollination A × H are compared with those of cross-pollinations A × A or A × P, the presence of factors other than nutrition becomes evident. The effects of seasons, of day of anthesis, of individual pistillate plants, and of nutrition are all intermingled in their modification of immediate effects of pollen, so that it is impossible to rank them in the order of their importance.

SUMMARY

Strains of cotton inbred from 11 to 20 years or more were used to test the immediate effects of pollen on boll characters. Emasculated flowers of Acala cotton were pollinated with (1) Pima pollen, (2) Acala pollen, and (3) Hopi pollen, the Acala pollen having been taken from plants of the same inbred strain that comprised the pistillate plants receiving all three cross-pollinations. Significant differences among means for the several cross-pollinations were obtained

in number of seeds per boll, in seed index, in lint index, in fiber length, and in boll-maturation period.

It is shown that effects upon the expression of these characters by differences between (1) different years, (2) different days of anthesis in the same year, and (3) different individual plants used as pistillate parents are so great as conceivably to mask completely the effects, if any, of different pollens. The difference between means for different years approximated the average difference between means for the several cross-pollinations in all characters except number of seeds per boll. In this character the difference, although highly significant, was smaller than the minimum difference between means of cross-pollinations. In both seasons the maximum difference among means for successive days of anthesis and among means for each of the individual pistillate plants exceeded the maximum difference among means of the several cross-pollinations.

An assumed nutritional factor was shown to affect the expression of seed index, of fiber length, and probably of lint index. This suggests that a difference among the several pollens in ability to fertilize a high percentage of the ovules was another factor that determines the effects of cross-pollinations.

The influence of these various factors, and undoubtedly of others not covered by this study, are so intermingled that it is impossible to rank them in the order of their importance.

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CHLORATE TOXICITY AND PERSISTENCE IN RELATION TO SOIL REACTION[1]

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INTRODUCTION

There is evidence from various sources of lesser toxicity of chlorate in alkaline solutions. Thus Offord and d'Urbal (*17*)[2] reported that sodium chlorate was more toxic to *Nitella* in acid solutions than in neutral or alkaline ones. They used as their criterion of toxicity the time required for the outward diffusion of 50 percent of the vacuolar chloride, and found it to be correlated with the pH value of the solution. At pH 4.8, the time required was 38 hours; at pH 5.2, 56 hours; at pH 5.8, 81 hours; at pH 6.6, 87 hours; at pH 7.4, 120 hours; and at pH 8.2, 125 hours. In agreement with this relation, Yamasaki (*20*) found that potassium chlorate was most injurious to wheat and rice seedlings in solutions made acid and least injurious in those made alkaline. Hulbert, Bristol, and Benjamin (*10*) discovered that sodium chlorate wilted cut plants most rapidly in acid solutions (pH 6.0 or below), but they were unable to show with certainty that the toxicity of chlorate sprays was enhanced by acidification. However, other field tests (*4*, *7*, *16*) have proved that chlorate sprays are most effective as herbicides when made slightly acid. And finally Grau (*9*) found that the application of lime to soil retarded the killing action of chlorate and apparently somewhat increased the length of time the chlorate was effective.

The purpose of the present investigations was to determine the importance of soil reaction, first as a factor in the effectiveness of sodium chlorate added to the soil for weed control, and secondly as a factor in the persistence of the chlorate in treated soils.

MATERIAL AND METHODS

Water-culture experiments were carried out with seedlings of soybean (Palmetto), sunflower, and barley (Hannchen) grown in acid, neutral, and alkaline solutions containing magnesium sulfate, calcium nitrate, ferric citrate, and mono-, di-, and tribasic potassium phosphate in such proportions as to buffer the solutions at the desired reactions. Acidity was checked colorimetrically every day or every other day, and in the first experiments was maintained by frequent change of solutions. Later the reaction of the acid cultures was adjusted as necessary by adding a little phosphoric acid, and that of the alkaline ones by adding sodium hydroxide or a small amount

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[2] Italic numbers in parentheses refer to Literature Cited, p. 493.

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of saturated sodium bicarbonate solution. Nitrogen concentrations were relatively low, 37 p. p. m., thus enhancing the toxicity of the chlorate (*8*, *13*).

The seedlings were germinated in quartz sand and transferred to the nutrient solutions when about 6 cm. high. Relative degrees of injury were estimated from leaf symptoms and from degrees of stunting.

Soil experiments with corn (*Zea mays*), sorgo (*Sorghum vulgare*), millet (*Setaria italica*), soybean (*Soja max*), sunflower (*Helianthus annuus*), and barley (*Hordeum vulgare*) were carried out with a composted Keyport clay loam. The greenhouse benches were divided by means of board partitions into plots about 3 feet square (surface area 10 square feet) each of which contained about 400 pounds of soil (325 pounds on an oven-dry basis). Some of the plots were made alkaline (pH 7.5–8.0) by the addition of slaked lime at a rate of 1, or in some experiments, 2 pounds, per plot. Others were acidified to near pH 5.2 with about 400 cc. of sulfuric acid, much diluted and sprinkled on the soil with a watering can. Others were left at the natural reaction, always approximately neutral. After the lime or sulfuric acid was thoroughly mixed with the entire mass of soil, sodium chlorate in solution was similarly well mixed into half of the plots of each reaction, the rest being left as controls. Fifteen grams of chlorate were added to each plot except in the case of the experiment with sunflower in table 3, where the quantity was half as much. By measuring the water applications throughout the experiments, soil moisture was kept fairly uniform and leaching avoided.

The acidity of the soil was determined at the beginning and end of each experiment. Approximately 100 gm. of soil were shaken with 100 cc. of water in a wide-mouthed bottle and allowed to settle overnight, or until the supernatant extract was clear. When the soil was unusually wet, the amount of water was reduced to 90, or in extreme cases, to 80 cc., so that the ratio of water to weight of soil was always approximately 1 to 1. Five-cubic-centimeter samples were carefully pipetted from the clear water layer for colorimetric determination of pH by means of the Taylor slide comparator.

To obtain plants as uniform in size as possible, extra seeds were sown and the smaller seedlings discarded soon after germination. The same number of plants of a given crop was always left in each row of the plots to be compared.

In order to correct for differences in size that might be caused by differences in the soil reaction, degrees of chlorate injury are indicated in the tables by the magnitude of the so-called growth percentage, i. e., the average green weight of the tops of an entire culture or plot expressed as a percentage of the corresponding weight for control plants in a corresponding culture or plot without chlorate. Relative amounts of injury were also apparent from the degree of leaf discoloration, for chlorosis and, with severe injury, necrosis were most severe on the smaller, more stunted plants. Wherever growth percentages were near 100, indicating normal size, leaf color was also normal. Absolute weights of the control plants are given in the columns to the right in each table in order to show the effect of the soil reaction itself. The recording of these weights also makes possible calculation of the absolute weights of the chlorate-injured plants.

RESULTS

CHLORATE TOXICITY IN RELATION TO ACIDITY

Symptoms of injury by chlorate were distinct and easily recognized on all the crops. Chlorosis, with brown spots that often became necrotic, was characteristic, and the lower leaves died. On barley there was a grayish withering of the leaf tips, extending eventually, in severe injury, to the entire leaf. In water cultures these symptoms appeared most quickly if the plants were allowed to develop a good root system before being transferred to solutions containing chlorate; so the procedure was adopted of allowing them to grow for a week, after germination in sand, in a solution having a favorable, approximately neutral reaction. Control plants grown without chlorate always prevented confusion of symptoms due to chlorate with those due to an unfavorable reaction of the solution.

The controls showed that near-neutral and alkaline solutions were most favorable for growth of soybean and sunflower, respectively, acidity as high as pH 5.0 being highly injurious. Barley grew quite uniformly in all the solutions, tolerating acidities as high as pH 5.0 much better than either soybean or sunflower. Some chlorosis from iron deficiency occurred occasionally in alkaline solutions, but it was not serious in the 2-week period of the experiments.

Several preliminary experiments with wheat showed no effect of the solution reaction on chlorate toxicity. But subsequent trials with soybeans grown under similar conditions showed definitely that the leaf symptoms produced by 25 p. p. m. of sodium chlorate were most severe where the nutrient solution was below pH 6.0, less severe where it was between pH 6.0 and 7.0, and slight or absent between pH 7 2 and 8.3. Sunflower also proved to be most severely injured by chlorate in the acid solution. Because of the poor growth of the control plants in the more acid solutions, subsequent tests were carried out with barley, which grew well over a wider range of reactions.

For the first of the experiments with barley, seedlings grown for a week in a near-neutral solution to develop uniform root systems were transferred to solutions containing 0, 10, 20, 40, and 50 p. p. m. of chlorate, respectively. Each series was replicated at acid (pH 5.1 6.2), neutral (pH 6.8 6.9), and alkaline (pH 9.1–8.3) reactions After 3 days there were distinct symptoms of chlorate injury in the acid and near-neutral solutions at all chlorate concentrations except the lowest, 10 p. p. m. In the alkaline solution no symptoms were detectable at this time at any chlorate concentration except the highest, 50 p. p. m. Two days later, injury had appeared here with 40 and 30 p. p. m. also, but to a much lesser degree than at these concentrations in the neutral and acid solutions. The contrasting appearance of the acid and alkaline series after 7 days is illustrated in figure 1. The control plants for the two reactions (extreme left in each series) look alike.

After 11 days the plants were cut, and those grown with 10 p. p. m. of chlorate at the acid, neutral, and alkaline reactions were photographed. Figure 2, *A*, shows that the chlorate injured the plants in the acid solution severely, those in the near-neutral one moderately, while those in the alkaline solution were apparently uninjured.

In another experiment the lower leaves of barley plants in the acid solution (pH 5.0–5.4) began to wither 2 days after the addition of 40

p. p. m. of chlorate to the cultures, whereas those in the neutral and alkaline solutions evinced no ill effects Two days later, when the photograph shown in figure 2, *B*, was made, the lower leaves of the plants in the neutral solution (pH 7.0–6.6) were also withered, but

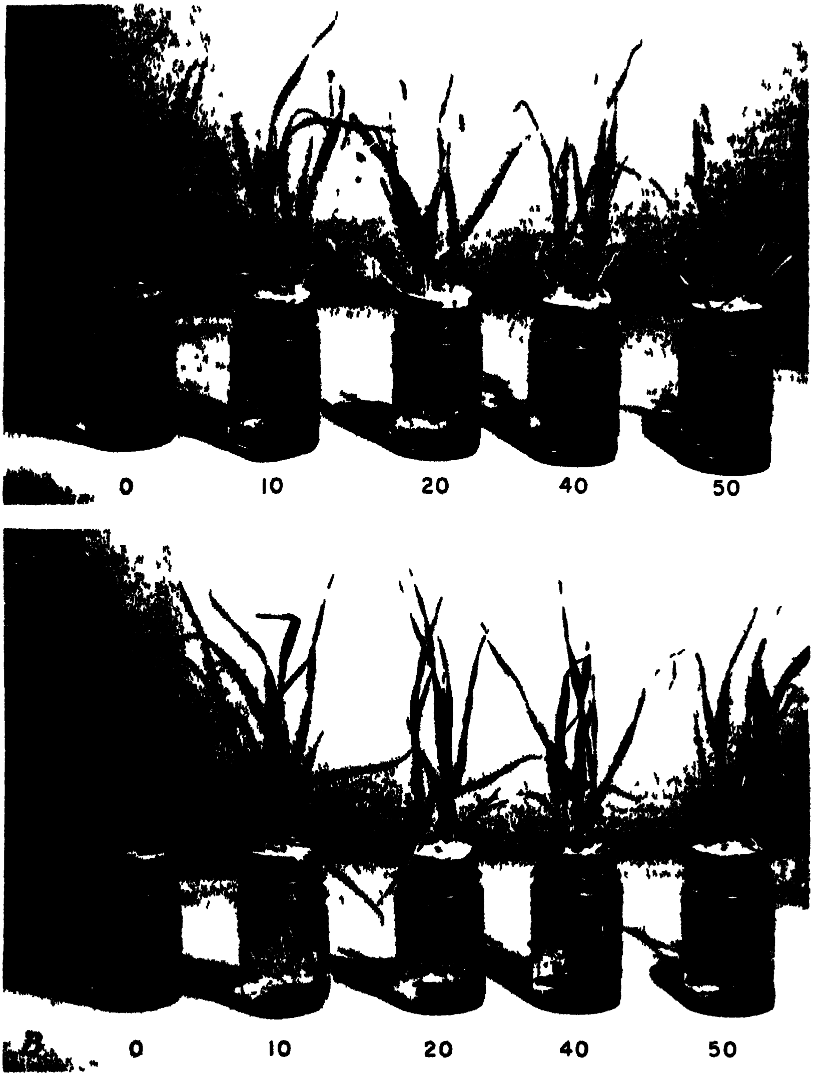

FIGURE 1.—Barley plants after 7 days in nutrient solutions containing 0, 10, 20, 40, and 50 p. p. m. of sodium chlorate *A*, In acid solutions, pH 5.1–6.2; *B*, in alkaline solutions, pH 9.1–8.3.

those in the alkaline solution (pH 7.9–7.4) were still practically uninjured, only the tips showing slight withering. Growth of the control plants without chlorate remained good at all three reactions for the duration of the experiments and showed that the symptoms attributed to chlorate were not due to unfavorable acidity in any case.

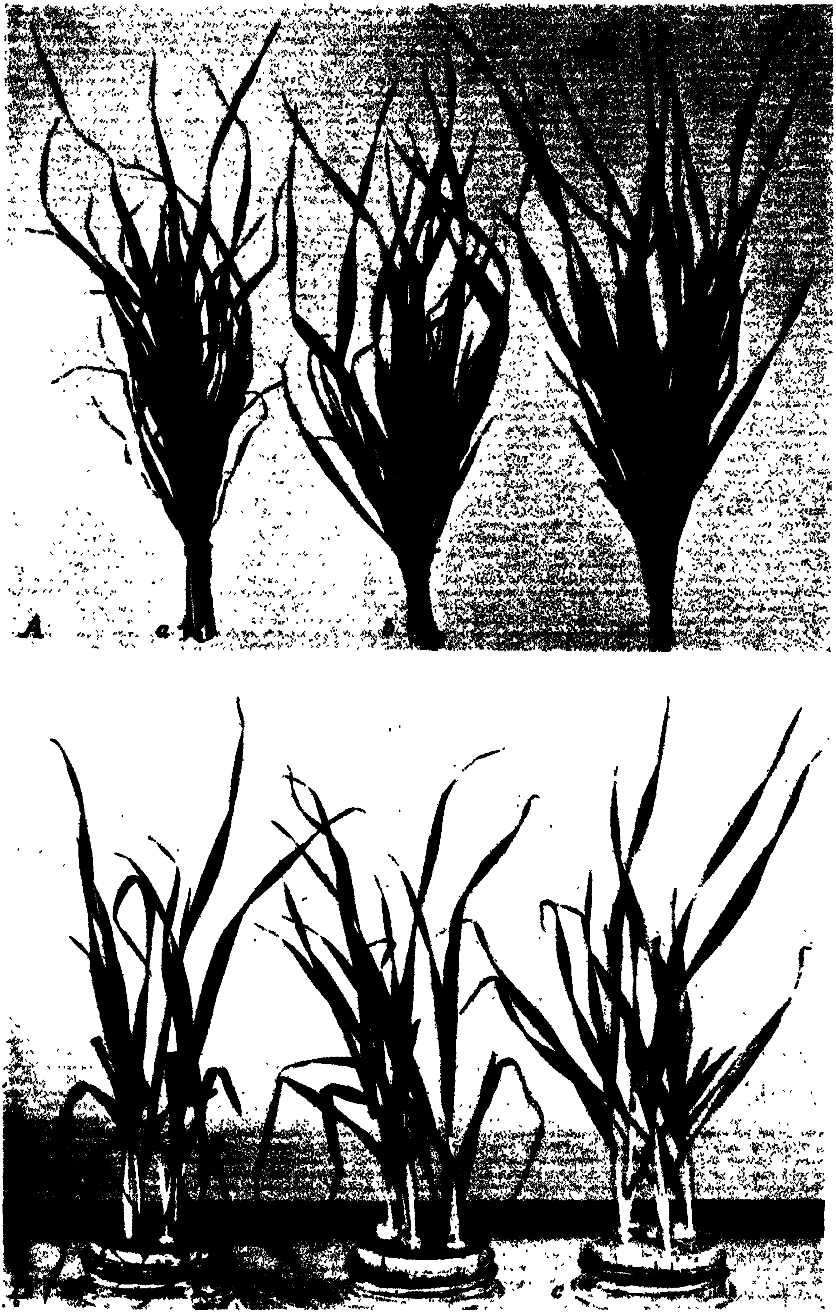

FIGURE 2.—*A*, Barley plants 11 days after the addition of 10 p. p. m. of sodium chlorate to: (*a*) Acid (pH 5.1–6.2), (*b*) neutral (pH 6.8–6.9), and (*c*) alkaline (pH 9.1–8.3) solutions. *B*, Barley plants 4 days after the addition of 40 p. p. m. of sodium chlorate to: (*a*) Acid (pH 5.0–5.4), (*b*) neutral (pH 7.0–6.6), and (*c*) alkaline (pH 7.9–7.4) solutions.

Another experiment with barley was carried out similarly with 20 p. p. m. of sodium chlorate in three solutions whose reactions ranged from pH 5.2 to 5.9, 6.4 to 7.0, and 9.2 to 8.1, respectively. After 4 days, only the plants in the acid solution showed chlorate injury—fired leaf tips and shriveled lower leaves. After 6 days, injury was severe in the acid solution, moderate in the near-neutral solution, and absent in the alkaline one. After 2 weeks, when the plants were cut and weighed, those in the alkaline solution averaged 96.5 percent as large as the corresponding controls without chlorate, as compared with 80 and 66.5 percent as large in the case of the plants in the neutral and acid solutions, respectively.

In the first soil experiment 3-week-old plants from duplicate plots of corn, soybean, millet, and sorgo all showed least chlorate injury in alkaline soil (pH 8). Other plants cut from the plots 2 weeks later gave similar results for each crop except millet, which at this cutting showed no difference between the degrees of chlorate injury in the limed and unlimed plots.

The large quantity of lime used here to produce a soil reaction of pH 8.0 was itself injurious, as shown by the smaller size of the control plants. Therefore, the experiment was repeated with half as much lime (1 pound per plot), which produced a soil reaction of pH 7.5. Again corn, soybean, sorgo, and barley were all less injured by the chlorate in the limed than in the unlimed plot at both of two cuttings, comprising alternate plants cut after 2 and 4 weeks, respectively. But in a repetition of the experiment immediately afterwards, with millet, sorgo, and sunflower, this effect of the lime was not obtained. Thus, while liming generally rendered the chlorated soils less toxic, there were unexplained exceptions. Moreover, in no case was the tendency of alkalinity to reduce the toxicity of chlorate as pronounced in soil as it had been in the water cultures. It should be emphasized that each of the soil experiments was begun with newly chlorated soil in an effort to avoid complications that would ensue if the rate of decomposition of the chlorate should be affected by the soil reaction.

PERSISTENCE OF CHLORATE TOXICITY IN RELATION TO SOIL REACTION

It was observed in one of the experiments that barley plants of a certain limed plot were taller and less chlorotic than those of an adjacent neutral plot throughout their early seedling stage, but that they lost this advantage by the time they were cut and weighed at the age of 6 weeks. Their initial superiority reflected the lesser toxicity of chlorate in an alkaline substrate. The fact that as time went on the plants became increasingly poorer than those in the neutral plot suggested an inhibiting effect of the soil's alkalinity on the rate of decomposition of the chlorate, which would accord with Yamasaki's (*20*) finding that chlorates undergo reduction more easily in acid than in neutral or alkaline substrates.

To test the relative rates of recovery of acid, neutral, and alkaline soils made toxic by sodium chlorate, some greenhouse-soil plots were acidified to pH 5.2 with sulfuric acid, others were made alkaline (pH 8.0) with hydrated lime, and still others were left at the natural reaction, pH 6.8. Sodium chlorate was then stirred into half of them, and the rest left as controls without chlorate. Black Amber sorgo was sown the next day. Each series of plots was duplicated in two greenhouses designated A and B.

Five successive crops of sorgo were grown in these plots without further treatment. The average green weights of the plants of each of the five crops, expressed as percentages of corresponding averages for control plants in soil of the same reaction without chlorate, are given in table 1.

TABLE 1.—*Comparative persistence of chlorate toxicity*[1] *in acid, neutral, and alkaline soils*[2] *as shown by growth percentages*[3] *for successive crops of sorgo*

[Chlorate added to soil on July 24, 1939]

Sowing No.	Period of growth 1939–1940	Greenhouse	Growth percentages of chlorated plants at the following soil reactions			Average weights of control plants without chlorate at the various soil reactions		
			Acid	Neutral	Alkaline	Acid	Neutral	Alkaline
			Percent	*Percent*	*Percent*	*Grams*	*Grams*	*Grams*
1	July 25 to Aug. 23	A	2.3	1.8	3.5	23.11	20.11	13.09
		B	1.5	1.3	3.3	19.72	19.64	13.73
2	Aug. 31 to Oct. 5	A	25.1	21.3	8.8	9.04	9.29	8.40
		B	22.4	19.5	10.7	14.80	13.32	9.54
3	Nov. 10 to Dec. 19	A	65.9	64.5	26.3	1.59	1.66	1.74
4	Dec. 20 to Jan. 22	A	102.9	106.1	64.0	.55	.50	.48
5	Jan. 22 to Mar. 7	A	135.9	106.2	51.4	1.28	1.61	1.40

[1] The toxicity of all the chlorated plots was reduced between the first and second sowings by adding with thorough mixing 80 pounds of untreated soil to that in each compartment. The control plots received similar additions.

[2] The pH values ranged over the period of the experiment from 5.2 to 5.3, 6.8 to 6.6, and 8.0 to 7.5 for the acid, neutral, and alkaline soils, respectively.

[3] Average green weights of 80 to 100 plants in terms of corresponding weights of control plants from soil of the same reaction without chlorate as 100.

The data show that chlorate toxicity was initially very severe in all the plots, reducing the plants to a small fraction of the size of the controls. Those in the alkaline soil were the least injured, as shown by somewhat higher growth percentages. In successive croppings toxicity decreased progressively in all the plots, but much less rapidly in the alkaline ones than in the others; with the result that, where the plants were least injured in the first sowing, they were definitely the most injured in all subsequent ones. When the experiment was discontinued, after five sowings, the soil of these alkaline plots was still highly toxic, while that of the neutral and acid plots had lost its toxicity completely.

The weights of the control plants showed that the lime was itself injurious for the first two sowings but not thereafter. There was no significant difference in the size of the control plants of the neutral and acid plots at any time. The decline in yield with time is without significance for the present study, being due to the decreasing size attained by plants of similar age as the season progressed from summer through the winter months.

The soil plots in greenhouse B of table 1 were not resown with sorgo after the second sowing but instead were used for a series of five successive sowings of sunflower. Each plot was divided into two sections by a board partition, and sodium nitrate, at a rate of 150 gm. to 250 pounds of soil, added to one and not the other. The comparative persistence of chlorate toxicity in the various plots is shown in table 2.

TABLE 2.—*Comparative persistence of residual chlorate toxicity in acid, neutral, and alkaline soils*[1] *as shown by growth percentages*[2] *of successive sowings of sunflower*

[Chlorate added to soil on July 24, 1939]

Soil and sowing No.	Period of growth 1939-40	Growth percentages of chlorated plants at the following soil reactions: Acid	Neutral	Alkaline	Average weights of control plants without chlorate at the various soil reactions: Acid	Neutral	Alkaline
		Per cent	*Per cent*	*Per cent*	*Grams*	*Grams*	*Grams*
Without excess nitrate:							
1	Nov. 9 to Dec. 20	51.3	28.3	14.1	45.1	44.5	28.8
2	Dec. 20 to Mar. 6	58.5	40.4	11.9	44.0	49.3	36.2
3	Mar. 7 to Apr. 15	62.2	54.4	17.6	17.0	17.7	16.0
	Mar. 7 to Apr. 29	77.1	62.5	15.5	48.4	50.4	46.2
4	Apr. 29 to May 24	106.9	76.2	27.4	18.4	20.5	22.2
	Apr. 29 to June 3	109.3	83.2	25.4	48.4	51.8	52.0
5	June 4 to June 28	123.1	108.3	49.3	12.9	12.5	13.0
	June 4 to July 8	106.9	95.8	43.8	47.5	54.9	48.0
With excess nitrate:							
1	Nov. 9 to Dec. 20	58.3	59.4	23.1	35.1	30.2	29.7
2	Dec. 20 to Mar. 6	63.6	83.0	42.0	42.7	34.0	32.8
3	Mar. 7 to Apr. 15	64.2	74.6	38.9	14.2	13.8	13.0
	Mar. 7 to Apr. 29	83.0	78.0	32.2	43.8	41.8	41.0
4	Apr. 29 to May 24	86.2	72.9	36.8	21.2	20.8	20.0
	Apr. 29 to June 3	82.2	90.1	37.0	47.7	46.7	46.7
5	June 4 to June 28	103.4	96.8	57.5	12.4	11.9	12.1
	June 4 to July 8	86.5	115.7	45.3	51.8	45.3	[illegible]

[1] The pH values obtained over the period of the experiment ranged from 5.3 to 5.5, 6.8 to 7.2, and 7.5 to 7.6 for the acid, neutral, and alkaline soils, respectively.

[2] Average green weights of about 20 plants in terms of corresponding weights of control plants from soil of the same reaction without chlorate as 100.

Much the smallest growth percentages, indicating greatest toxicity of the chlorate, were obtained for plants of the alkaline plots throughout. The failure of these plants to show an initial superiority is accounted for by the fact that the chlorate had already been in the soil for 3.5 months (since July 24) at the time of the first sowing, so that differential decomposition of the chlorate had already occurred—greatest in the acid, least in the alkaline soil. Toxicity disappeared completely from the acid and neutral soils by the time of the fifth sowing, but the plants in the alkaline plots were still very chlorotic at this time and were only about half the size of the control plants in the same soil without chlorate. The data show conclusively that lime greatly increased the time required for decomposition of the chlorate. The contrast in the appearance of the plants of the limed and unlimed plots by the time of the third sowing is illustrated in figure 3.

When the percentages for plants from the nitrated plots are compared with those from the plots without added nitrate it is seen that the effect of nitrate in reducing toxicity, first discovered by Crafts (8), was more pronounced in the neutral and alkaline soils than in the acid one. As a result, the plants of the acid plots were not superior to those of the neutral plots in the nitrated soil, thus constituting the only exception to the generalization of most rapid decomposition of the chlorate in the most acid soils.

Other experiments with sunflower and barley are summarized in table 3, both begun with newly chlorated soil.

The plants of the first sowings of both crops were somewhat less injured by chlorate in the alkaline plots than in the neutral ones. Subsequently this relation was reversed, as in the experiment of table

FIGURE 3.—Comparative degrees of chlorate chlorosis and stunting of 5-week-old sunflower plants grown in (*a*) limed, (*b*) neutral, and (*c*) acidified soils treated 9 months previously with equal quantities of sodium chlorate. The control plants, without chlorate, showed no effects of the soil reaction, all being normal in size and color at this time.

1 where, as here, the seed was sown within a few days after the application of chlorate. During the several-month period of the experiments, chlorate toxicity disappeared completely from the acid soils, and was much reduced in the neutral soil, but it changed much more slowly in the alkaline one. Concomitantly, leaf chlorosis, equally severe on all the plants at first, disappeared completely from the acid plots but persisted with almost unabated severity in the alkaline plots to the end. Plants of the neutral plots were clearly intermediate after the first sowings. The color of all the control plants was normal

TABLE 3.—*Comparative persistence of chlorate toxicity in acid, neutral, and alkaline soils* [1] *as shown by growth percentages* [2] *of successive croppings of barley*

[Chlorate added to soil on Jan. 2 and Mar. 11, 1940, for experiments with barley and sunflower, respectively]

Crop and sowing No.	Period of growth	Growth percentages of chlorated plants at the following soil reactions: Acid	Neutral	Alkaline	Average weights of control plants without chlorate at the various soil reactions: Acid	Neutral	Alkaline
		Per cent	*Per cent*	*Per cent*	*Grams*	*Grams*	*Grams*
Barley:							
1	Jan. 3 to Feb. 14	12.6	8.9	13.4	4.07	3.8	3.89
2	Feb. 16 to Apr. 1	15.3	7.9	7.2	11.27	11.4	10.70
3	Apr. 1 to Apr. 30	40.8	21.9	17.9	2.75	2.86	2.94
4	Apr. 30 to May 23	81.9	53.8	33.1	2.30	2.08	2.43
5	May 23 to June 21	140.0	76.9	52.1	2.25	2.98	3.14
Sunflower:							
1	Mar. 12 to Apr. 5	50.3	36.9	41.6	8.49	7.07	4.98
	Apr. 8 to May 3	73.0	50.4	41.0	11.11	10.32	6.70
	May 3 to May 27	99.7	77.7	17.8	12.60	11.98	8.63

[1] The pH values obtained over the period of the experiments ranged from 5.3 to 5.7, 6.8 to 7.0, and 8.1 to 7.6 for the acid, neutral, and alkaline soils, respectively.

[2] Average green weights of 75 to 85 plants of sunflower and at least 100 of barley in terms of corresponding weights of control plants from soil of the same reaction without chlorate as 100.

There is a further relation that should be pointed out in connection with the first sowings of tables 1 and 3 (comprising all the experiments begun with newly chlorated soil), namely, that the plants of the acid plots were not the most injured, as would be expected, but were less injured than those of the neutral plots in every case. This disagreement with the direct relation that obtained in the water cultures is probably attributable to the effect of acidity on decomposition of the chlorate by reducing agents of the soil, superimposed on the direct effect of acidity on chlorate injury to the plant. This point will be discussed later.

The occurrence of growth percentages well above 100 for final sowings, such as those of the acid plots of tables 1 and 3, show that after chlorate toxicity had disappeared the plots produced larger plants than did the adjacent control plots of the same reaction without chlorate. Several investigators have reported such instances of apparent stimulation of crops by chlorate. Strobel and Scharrer (*19*) and Bissey and Butler (*5*) have observed it with wheat, and Bowser and Newton (*6*) with flax, although the latter attributed it to elimination of weeds by the chlorate. Stimulation of sorghum was observed by Timmons (unpublished report, 1939) who stated (p 20) that "there was a definite tendency for the yields of most of these crops [sorghums] to be higher on the treated areas than on check plots, probably due to the moisture conserved by the absence of growth on the treated areas the previous year or two." Conservation of nutrients would also be expected where chlorate has previously reduced plant growth. Bissey and Butler (*5*), however, found that the apparent stimulation observed by them did not result from reduction of fertility in the check plots, for these remained equally productive over the period of experimentation. In the present investigation relatively greater exhaustion of the soil in the control plots by the end of each experiment can be assumed but it cannot be proved from the data available. It seems to the writer that another explanation may be found in a possible destructive effect of chlorate on the deleterious soil organisms to which crops like sorghum are particularly susceptible.

CELL-SAP ACIDITY AND SUSCEPTIBILITY TO CHLORATE INJURY

The effect of the reaction of the substrate on chlorate toxicity suggested the possibility of a relation between plant acidity and degree of susceptibility to chlorate injury which varies widely with different crops (*12*). Although reports found in the literature (*11*) showed no acidity differences that could be correlated with differences in susceptibility to chlorate, it seemed desirable to compare the acidity of some of the crops when grown side by side in the same greenhouse.

Some 200 pH determinations[3] on the expressed juice of leaves of 15 different crop plants were made with a Leeds and Northrup glass-electrode apparatus checked frequently with standard buffer solutions. A comparison of these determinations showed that differences in susceptibility to chlorate injury cannot be explained on the basis of differences in plant acidity. Thus, pH values for four varieties of barley, one of the most susceptible crops, ranged from 5.91 to 6.27, as compared with a range of 5.88 to 6.05 for four varieties of oats, a highly resistant crop; and pH values for spinach, which generally proved most susceptible of all to chlorate, ranged from 5.84 to 6.24, as compared with a range of 5.80 to 5.89 for the highly resistant flax. Obviously some factor other than sap acidity is responsible for the differences in the severity of chlorate effects on these plants.

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[3] These determinations were made by A L Pitman, of the Industrial Farm Products Research Division Bureau of Agricultural Chemistry and Engineering, U S Department of Agriculture

DISCUSSION

The toxicity of a given concentration of chlorate tends to vary directly with the hydrogen-ion concentration of the substrate. However, from the rather small effects and the occasional exceptions obtained in the soil experiments of the present investigations it seems likely that the relation is of minor importance for the problem of chlorate treatment of soils for weed control. More important seems the fact that chlorate toxicity disappears so much more slowly from limed soil than from near-neutral and acidified soils. This finding, if confirmed under field conditions, would indicate a distinct disadvantage in the use of chlorate on alkaline soil, for to the tendency of the chlorate application to be least toxic, hence least effective in killing the weeds, is added the even more serious objection of subsequent slower recovery of such soils for succeeding crops. Presumably the explanation of the greater persistence of chlorate toxicity in limed soil is to be found in the relatively slow decomposition of the chlorate at alkaline reactions.

Whenever an experiment was begun with soil to which chlorate had been added several months previously (in which presumably the differential decomposition due to soil reaction would already have occurred) the data always failed to show the initial superiority of chlorated plants in limed soil that would be expected from the fact of their superiority in alkaline nutrient solutions.

The plants of acid soil plots were less injured by chlorate than were those in neutral plots. This reversal of the direct correlation of toxicity with degree of acidity, found to obtain in water-cultures of both the present and previous investigations (*10*, *17*, *20*), may be explainable on the basis of more rapid decomposition of chlorate in the acid soil –a reaction not occurring in culture solutions of similar acidity because of lack of reducing agents. As stated by Offord (*16*), "The high oxidizing potential of sodium chlorate and the readiness with which this compound gives up its oxygen when intimately associated with organic matter is a well-known chemical fact."

In explanation, then, of the relation between soil reaction and persistence of chlorate toxicity, it can be assumed that the rate of decomposition of chlorate to the relatively harmless chloride, a process known to occur in the soil and to be affected by various conditions (*2*, *3*, *6*, *9*, *14*), is favored by acidity and hindered by alkalinity, as might be expected from its chemical properties (*15*, *p. 313*). In accord with this explanation is Yamasaki's finding (*20*) that glucose and aldehydes can reduce chlorates when the solutions are acid but not when they are neutral or alkaline. Since one of the advantages in the use of chlorates for weed control is their relatively rapid disappearance from treated soils, their persistence under alkaline conditions would indicate the need of caution in applying them to limed soils, and possibly to the alkaline soils of the western part of the United States. Certainly, to apply lime in order to hasten recovery of the soil after chlorate has been used to kill weeds, an idea that had been suggested by the tendency of chlorate to be least toxic in alkaline solution cultures, can now be expected to greatly prolong the toxic condition. The best recovery of an alkaline soil by the time all toxicity had disappeared from adjacent acid plots was to 64 percent of normal (table 1). Usually recovery was to about 50 percent of normal.

Incidentally, it is of interest to note in this connection Yamasaki's idea (*20*) that the active agent in chlorate toxicity is the hypochlorite ion produced on reduction of the chlorate to hypochlorite within the plant. He reports that susceptibility to chlorate is actually greatest in those plants that are highest in such reducing substances as glucose and aldehydes. If the reduction takes place only in acid solutions, it might be assumed that the more acid the plant the greater its susceptibility to chlorate. Yamasaki actually found that growing plants in acid solutions increased their susceptibility to chlorate and assumed the effect to be due to an increase in their sap acidity. However, he found no correlation between the acidity measurements for seedlings grown under ordinary conditions and their relative susceptibility to chlorate injury. Nor could any such correlation be found in the present investigation.

Arenz' finding (*1*) that barley is high in reducing capacity, as compared with lupine, should be noted in this connection, for barley is one of the most susceptible crops to chlorate. Also of interest, in view of the extreme susceptibility of spinach, is Stoklasa's finding (*18*) that the Chenopodiaceae in general are high in oxalic acid. However, the hydrogen-ion concentrations obtained by Stoklasa, the present writer, and others (*11*) for the expressed juice of spinach are no higher than those of more resistant crops.

The assumption is made throughout this paper that the effect of lime on the persistence of toxicity of chlorate-treated soils is an effect of alkalinity. However, a puzzling aspect presents itself in the fact that so large an effect persists in the limed soil after its alkalinity has decreased through successive sowings to values as low as pH 7.5, as in the experiment summarized in table 1. Furthermore, it is surprising that there should be such an extreme contrast in the toxicity of chlorate in soils whose reaction differed no more than did that of the "neutral" and alkaline soils of table 2, the former ranging, between the beginning and end of the experiments, from pH 6.8 to 7.2 and the latter from pH 7.5 to 7.6. There is a suggestion here that the action of lime in delaying the disappearance of chlorate toxicity from soils may be some effect other than that of alkalinity. On the other hand there is the fact that acidifying the soil could hasten the disappearance of toxicity as outstandingly as liming delayed it, which would point to degree of acidity as the determining factor. Certainly such pronounced and regular trends of declining growth percentages with decreasing degrees of soil acidity as those shown by table 3 would seem to justify such interpretation.

SUMMARY

The toxicity of sodium chlorate to soybean, sunflower, and barley in water cultures generally varied directly with the acidity of the nutrient solution, being least at alkaline reactions and greatest at acid ones. Decreasing the soil acidity of greenhouse plots to about pH 8.0 with lime sometimes, but not always, decreased the toxicity of newly added chlorate.

Chlorate toxicity disappeared much more slowly from limed than from unlimed soil. In general, it disappeared most rapidly from acidified soil, but the difference between the rates of recovery of the acid and neutral plots was usually less marked and less consistent than the difference between these and the alkaline plots.

In consequence of the dual effect of acidity on the toxicity of chlorate and on its rate of decomposition—alkaline plots that were the least toxic immediately after chlorate was added were the most toxic to subsequent crops. This reversal of the initial relation between limed and unlimed plots is believed to be due to an effect of soil reaction on the rate of decomposition of the chlorate, a process hindered by alkalinity and favored by acidity.

Since a given concentration of chlorate tends to be least toxic in alkaline soil, hence a less effective poison for weeds, and at the same time is a more lasting poison for succeeding crops, a question is raised as to the advisability of applying it to alkaline soils.

There was no correlation between the pH value of the expressed juice of various crop plants and their relative susceptibility to chlorate injury.

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A COMPARISON OF FUSARIUM AVENACEUM, F. OXYSPORUM, AND F. SOLANI VAR. EUMARTII IN RELATION TO POTATO WILT IN WISCONSIN[1]

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INTRODUCTION

The fusarium wilts of potato (*Solanum tuberosum* L.), while of relatively minor importance to the potato industry as a whole, constitute a major problem in many local areas. Their endemic occurrence in certain regions and their sporadic appearance in others render them constant hazards to the potato crop. Appel (*1*),[2] working in Germany in 1907, described a leaf-rolling disease of potato which he believed to be due to one or more species of *Fusarium*. The disease characteristically produced a folding together of the margins of the leaflets, while the lower leaf surfaces of diseased plants presented a leaden appearance and the entire foliage frequently assumed a reddish tint. The potato wilt diseases associated with *Fusarium solani* (Mart.) App. & Wr. var. *eumartii* (Carp.) Wr. and *Fusarium oxysporum* Schlecht. *f.1* Wr. (*F. oxysporum* f. *tuberosi* S. & H.) (*19*) have been studied extensively and the literature on the subject reviewed fully by Goss (*5*, *6*, *7*, *8*, *9*, *10*).

A potato disease differing somewhat in symptoms from the common potato wilts has been observed in Wisconsin for several years. From infected plants collected in the summer of 1936 a species of *Fusarium* was isolated which differed in cultural characters and spore type from *F. solani* var. *eumartii* and *F. oxysporum*. This culture was identified as *Fusarium avenaceum* (Fr.) Sacc. by Dr. Otto A. Reinking of the New York Agricultural Experiment Station. Several phases of the disease have been studied and in each case comparison has been made with the two wilt diseases mentioned above. For convenience, the three diseases will be referred to herein as eumartii wilt, oxysporum wilt, and avenaceum wilt.

PATHOGENICITY STUDIES

Many isolations of *Fusarium avenaceum* and various other species of *Fusarium* were made from diseased plants collected in commercial fields of central and northern Wisconsin during the summer of 1936. Single-spore cultures were obtained by the dilution-plate method on clear agar. Four plants each of Katahdin and Bliss Triumph varieties were inoculated with each culture. The fungus was introduced

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[1] Received for publication December 2, 1940

[2] Italic numbers in parentheses refer to Literature Cited, p 524

by inserting mycelium into a cleft cut into the stem just below the ground level after the stem surface had been disinfected. Sterile nonabsorbent cotton was placed over the wound and the soil was replaced. Isolates which produced a progressive discoloration of the vascular elements some distance from the point of inoculation were retained for further study; the remainder was discarded. Repeated tests showed *F. avenaceum* to be parasitic on potato, while plants inoculated with other fungus isolates and the control plants remained healthy.

In the belief that a pathogen new to potato had been obtained a soil inoculation test was set up with *F. avenaceum*. A mixture of 3 parts of composted soil to 1 part sand was autoclaved for 2½ hours, along with the 6-inch clay-pot containers. After exposure to the air for one week the autoclaved soil was inoculated with 23-day-old corn-meal-sand cultures of *F. oxysporum*,[3] *F. avenaceum*, and *F. solani* var. *eumartii*.[3] Suberized seed pieces of Irish Cobbler were planted and five pots of each inoculation were placed at air temperatures of 18° and 24° C.

Plants infected from the soil by *F. solani* var. *eumartii* were dead in 7 to 10 days after emergence at 24° and in 14 to 20 days at 18°. The plants infected by *F. avenaceum* or *F. oxysporum* were less seriously affected, living on an average for 65 days after emergence. The plants affected by oxysporum wilt displayed symptoms of leaf yellowing for an average of 20 days before complete wilt and death occurred. Those affected by avenaceum wilt showed early, distinct signs of the disease 20 days after emergence; the plants recovered for a time but finally succumbed to the wilt at the same time as did those infected by *F. oxysporum*. The control plants in sterilized soil to which sterile corn meal and sand had been added were healthy 80 days after emergence, at which time the experiment was discontinued. The respective organisms were reisolated successfully in all instances from the lower stem, roots, or tubers of infected plants. These results indicated that the strain of *F. avenaceum* tested was capable of producing infection and discoloration of the stems, stolons, roots, and tubers from which the fungus could be reisolated.

SINGLE-SPORE STRAINS

The pathogenicity of 12 single-spore strains of *Fusarium avenaceum* from one of the original isolates was tested by stem-puncture inoculation and by the use of inoculated, sterilized soil. In the artificially infested soil several weak strains were able to cause root infection and browning, but were not capable of affecting the vascular tissues of the stem to any great extent. When these cultures were injected into cleft wounds in the stem, a vascular discoloration extending 1 to 1½ cm. above the point of inoculation was produced after 20 days No foliage symptoms were produced. Another group attacked the roots of plants in infested soil, invaded the lower stem, and produced in it a vascular discoloration extending 1 to 2 cm. above the point at which the infected roots were attached. Stem inoculations resulted in vascular penetration 2 to 3 cm. from the point of inoculation after 20 days. Mild foliage symptoms were detected on a few plants in this group. The third group of single-spore strains readily produced root infection and vascular infection of the stem from infested soil, and definite foliage symptoms were apparent. Vascular penetration extended 5 to 7 cm. above the point of inoculation by the stem-puncture method. Thus the 12 single-spore isolates tested produced three more or less distinct classes of infection as measured by the extent of root and stem penetration and the resulting foliage symptoms. Seven of the most virulent single-spore lines were combined for use in later inoculation studies.

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[3] Dr. R. W. Goss, University of Nebraska, kindly furnished subcultures of *F. oxysporum* and *F. solani* var. *eumartii* from isolates on which he reported in 1940 (*10*)

SEED-PIECE INOCULATION

Several methods of seed-piece inoculation were tested with *Fusarium avenaceum*, *F. oxysporum*, and *F. solani* var. *eumartii* in the greenhouse. Fifty seed pieces each of Irish Cobbler and Rural New Yorker were used for each treatment and planted at once in flats of sterilized soil. Uninoculated controls for each method were included. The methods were as follows:

Presuberized. The seed piece was cut and allowed to stand in a ventilated, saturated atmosphere for 4 days at 24° C. to allow suberization and cork formation to take place before inoculation; it was then dipped in a suspension of spores and mycelium of the fungus and planted.

Fresh cut.—Freshly cut seed tubers were dipped at once in the fungus suspension and planted immediately.

Plugged. A plug was removed from the seed piece; a disk of mycelium and agar was inserted; the plug was replaced and sealed with paraffin.

Cleft. A ¼-inch cleft was cut near the eye of the seed piece, mycelium was inserted with a needle, and the cleft was resealed.

Plants inoculated with *F. avenaceum* and *F. solani* var. *eumartii*, together with the uninoculated controls, were held at 24°; *F. oxysporum* inoculations were held at 28°.

The measure of success for each method was determined by recording the amount and degree of seed-piece and stem infection after 22 days when the plants were 8 to 10 inches high. Percentage of infection and disease-index values were calculated from these tabulations. The disease index is similar to that used by Walker, Larson, and Albert (*20*) in the analysis of potato scab data.[4] The cleft and plugged methods gave severe infection when *F. solani* var. *eumartii* was used and numerous plants failed to emerge or were killed soon after emergence. *F. avenaceum* and *F. oxysporum* failed to give consistent infection by these methods under greenhouse conditions. The results for the presuberized and fresh-cut methods are shown in table 1. Although the numbers were too small for satisfactory statistical treatment, there were definite differences between species, varieties, and the two methods of inoculation.

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[4] The method of calculation was as follows: Group-weight values were given for clean—0, for slight infection—1, for moderate—2, and for severe infection—5. These arbitrary values were allocated to represent the relative severity of the respective grades. The number of seed pieces or stems in each class was multiplied by its respective class weight, the products were then added and the total multiplied by 100. The figure obtained was divided by five times the total for the treatment to arrive at the disease index for that treatment, i. e., $\frac{\text{total of weighted values} \times 100}{\text{total for treatment} \times 5}$ = disease index. Disease index is, therefore, a combined measure which expresses in one value both the percentage and the degree of infection.

TABLE 1.—*The effect of two methods of inoculation with three species of Fusarium on seed-piece and stem infection in the greenhouse*

[Each figure represents 50 potato seed pieces]

Potato variety and species of *Fusarium*	Seed-piece infection				Stem infection			
	Presuberized		Fresh cut		Presuberized		Fresh cut	
	Percent	*Disease index*	*Percent*	*Disease index*	*Percent*	*Disease index*	*Percent*	*Disease index*
Irish Cobbler								
F. solani var. *eumartii*	20.0	7.6	84.0	29.6	9.1	5.4	37.4	19.2
F. avenaceum	21.0	7.2	24.0	11.2	6.9	3.0	14.6	4.4
F. oxysporum	20.0	15.8	38.2	23.6	5.5	1.6	27.1	10.6
Uninoculated control	2.0	.4	4.2	.8	0	0	0	0
Rural New Yorker								
F. solani var. *eumartii*	100.0	78.0	100.0	100.0	64.5	27.4	78.0	63.0
F. avenaceum	14.0	5.6	32.0	12.0	6.0	1.2	14.3	3.0
F. oxysporum	44.0	14.4	76.0	55.0	21.4	4.7	72.0	20.0
Uninoculated control	0	0	2.0	.4	0	0	0	0

A beneficial effect of suberization was seen on both potato varieties, although Rural New Yorker thus treated was more severely attacked than Irish Cobbler. The effect of suberization was evidenced by a decrease in severity, or by a delay in attack, together with a reduction in the number of plants affected. The "fresh-cut" inoculations generally allowed a greater amount and a greater degree of infection than did the presuberized treatment.

F. avenaceum and *F. oxysporum* produced consistent infection of seed pieces and stems, but both species were in general less infectious than *F. solani* var. *eumartii*. The most extensive and most severe infection was found with *F. solani* var. *eumartii* on Rural New Yorker variety, where the disease index more nearly approached the percentage values than was the case with the other species. A close relationship was noted between seed-piece rotting and stem infection. Reisolations from the stems of each lot yielded the respective organism used in inoculation.

These results are in general agreement with those of Goss (*5*, *6*) with *F. oxysporum* and *F. solani* var. *eumartii* and those of MacMillan (*13*) with *F. oxysporum*. The differences found between the three fungus species in the greenhouse experiments are consistent with the results observed later in field inoculations, although the same differences between the two varieties and the two methods of inoculation were not found in the subsequent field experiments

SYMPTOMS OF AVENACEUM WILT

The first symptom to appear on the foliage following infection by *Fusarium avenaceum* is a faint yellowing of the basal portions of the leaflets of the apical leaves. This stage is soon followed by more pronounced yellowing, approaching chlorosis, of the leaflets, together with a bunching of the leaflets and folioles due to a shortening of the internodes of the petiole. The apical leaves are reduced in size and there is a slight increase, under greenhouse conditions, in the number of leaflets and folioles. The leaves frequently assume a greyish cast and the texture becomes brittle and coarse as compared with that of the normal foliage.

The stages which follow vary to a large extent with different environmental conditions. With sufficient soil moisture and cool temperature, the affected plant continues to grow, and carbohydrates accumulate in the above-ground organs. Characteristic red or purple pigmentation occurs, together with an upward rolling of the leaves (fig. 1) and the production of aerial tubers in the leaf axils (figs. 2, 3). The plant at this stage resembles one suffering from rhizoctonia stem injury, while other combinations of stunting, yellowing, leaf rolling, and the presence of aerial tubers may produce a plant somewhat similar in appearance to haywire (*9*) of psyllid yellows (*17*). To distinguish between these diseases and avenaceum wilt on the basis of foliage symptoms alone is difficult; in this group, however, only avenaceum wilt consistently produces vascular discoloration of the lower portion of the infected stem.

FIGURE 1. Symptoms of avenaceum wilt showing the effect of inoculation with *Fusarium avenaceum* on Bliss Triumph potato at Madison, Wis Note the upward rolling of the leaves and wilting of the plant.

When low soil moisture and unfavorable high temperatures prevail, rapid wilting and death of the affected plant result sometimes, preceded by a gradual loss of chlorophyll and consequent yellowing of the foliage. Tipburn and loss of the lower leaves are common. Plants infected with *F. avenaceum*, whether grown in the greenhouse or field, are frequently indistinguishable from those affected with oxysporum wilt. This is particularly true where temperature and water supply are constant, or when high temperatures prevail. Inoculations in the field with *F. avenaceum* at Madison, Wis., occasionally produced slight red and purple coloration of the foliage accompanied by rolling of the leaves, but no aerial tubers were formed (fig. 1). In no case were these plants as striking in appearance as infected plants in a similar inoculation plot 200 miles north of Madison (figs. 2, 3) where purpling of the foliage and formation of aerial tubers were much more prevalent. Plants in this location affected with oxysporum wilt, and a few with eumartii wilt, displayed symptoms similar to those produced by *F. avenaceum*, but most of those affected with eumartii wilt

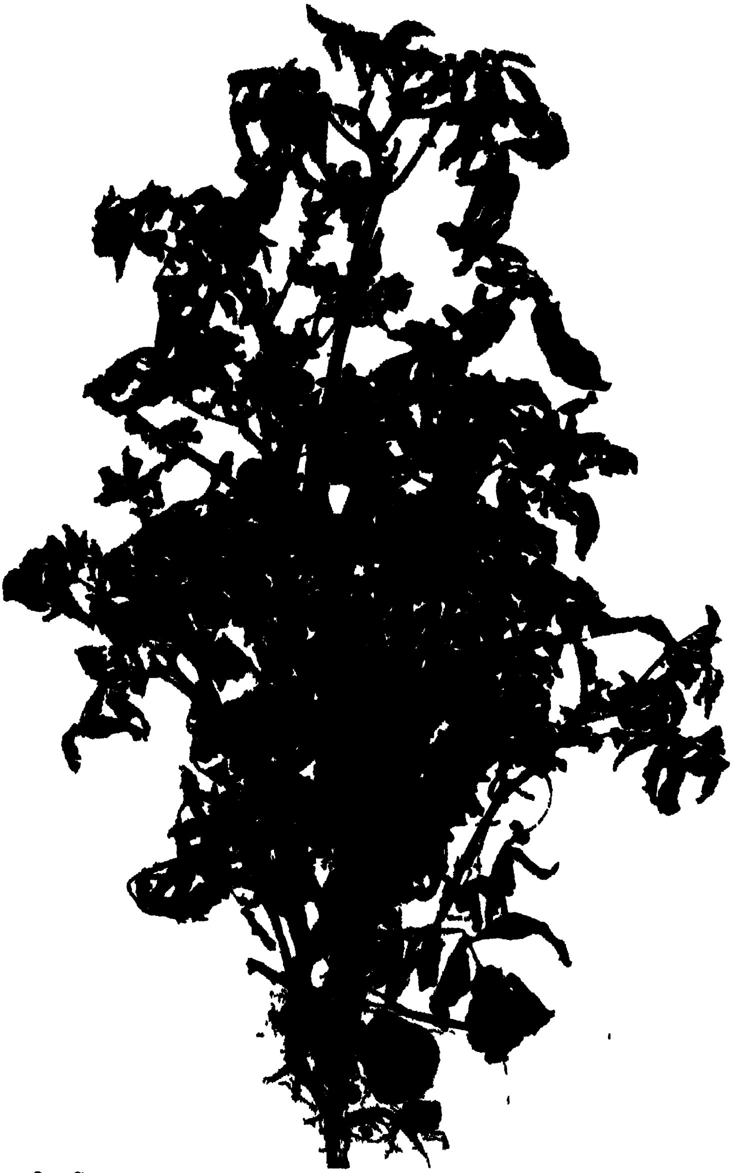

FIGURE 2.—Symptoms of avenaceum wilt showing the effect of inoculation with *Fusarium avenaceum* on Bliss Triumph potato at Starks, Wis. Note the upward rolling of the leaves and the axillary aerial tubers.

displayed traces of the interveinal mottle and bronzing characteristic of that disease.

The symptoms of avenaceum wilt described above are those most commonly found associated with mid- to late-season field infection in the northern half of Wisconsin. Severe early infection has been observed under field conditions, and especially after artificial inoculation. Plants affected thus either recover for a time, meanwhile showing partial masking of symptoms, or they succumb soon after emergence

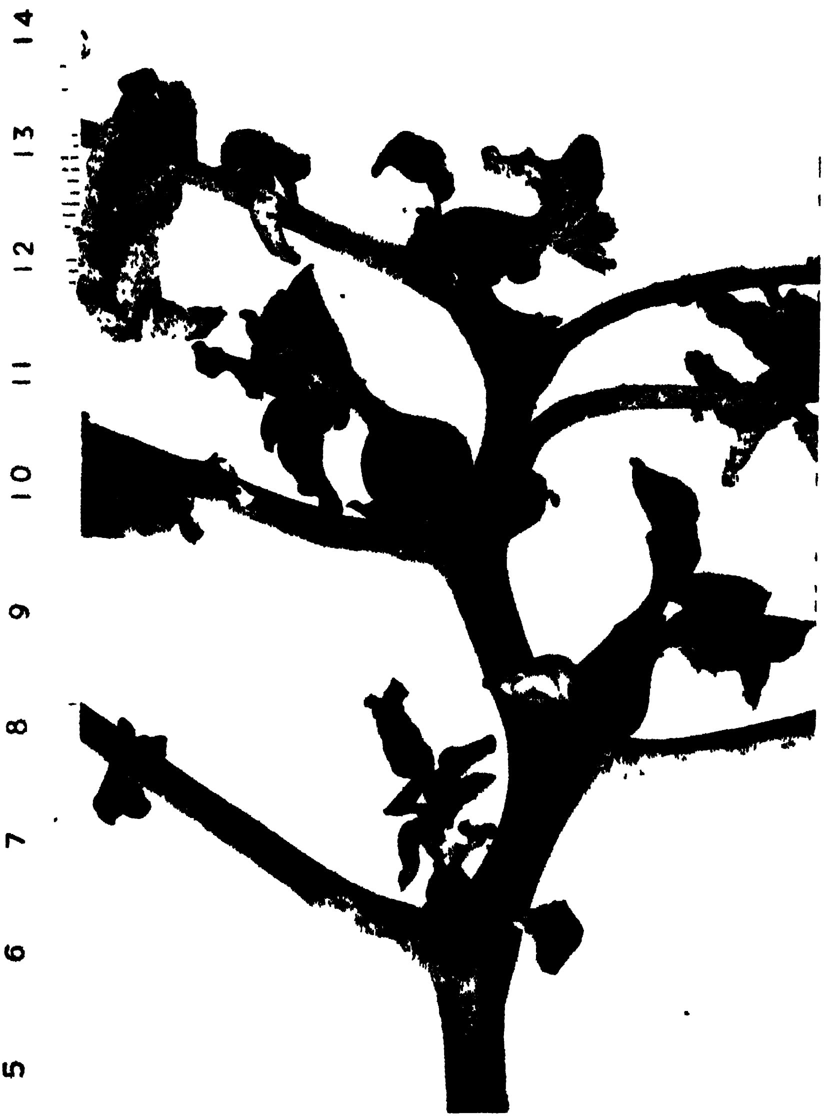

FIGURE 3.—The formation of aerial tubers in the leaf axils of a purple top potato plant infected by *Fusarium avenaceum* from the plot at Starks, Wis.

with a consequent reduction in stand and yield. Plants which are severely infected early in the season show stunting and bunching of the tops and a decrease in chlorophyll at the apex of the plant strikingly similar to yellow dwarf. However, infection by *F. avenaceum* does not result in discoloration of the pith like that in plants affected with yellow dwarf or eumartii wilt.

The roots and stems infected with *F. avenaceum* are indistinguishable from those affected by other fusarium wilts of the potato. Infection may develop unilaterally in the stem, or may be distributed throughout all vascular elements. Browning of the latter may precede fungal invasion or the two may be coincident. Discoloration has been observed to extend 6 to 8 inches above the soil level in full-grown infected plants, although the causal organism has seldom been isolated 3 inches above the soil. Pionnotes of the fungus were sometimes observed on the exterior of severely infected stems, slightly above the ground level. Actual fungal invasion, however, is more common in the below-ground parts. The infection progresses from the stem to the tuber through the stolon. Direct infection of the stolons or young tubers from the soil has not been observed. Seed-piece infection may occur, but the late-season effects of the disease and the ease with which root infection occurs indicate that the latter method of infection is the more common in Wisconsin. Vascular browning can likewise be traced macroscopically from an infected root into the stem xylem.

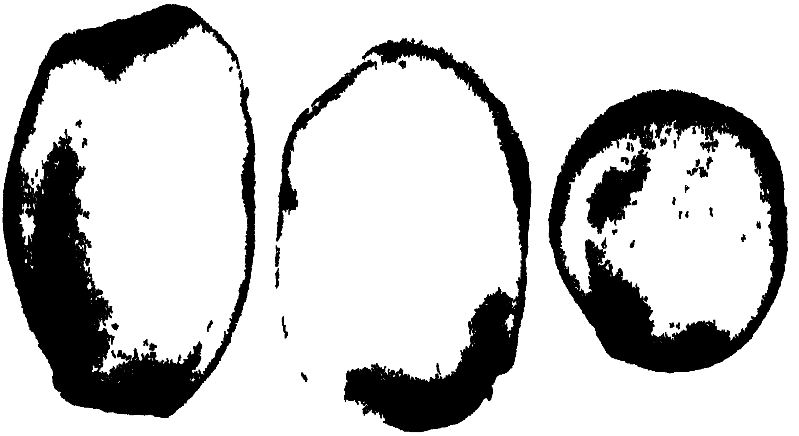

FIGURE 4 —Severely infected tubers from potato plants inoculated with *Fusarium avenaceum* showing vascular discoloration and necrotic spots in the cortex and pith. Photograph taken at the time of harvest

Affected tubers show a vascular discoloration which originates at the point of stolon attachment and extends into the tubers (fig. 4), varying among plants and among the tubers of an individual plant. Severely infected tubers may show a dry stem-end rot (fig. 5), which is externally discernible as a sunken area at the basal end of the tuber. This symptom differs from the water-soaked appearance of tubers affected by *F. solani* var. *eumartii* in that a characteristic powdery dry-rot is produced. The affected parts are gray to pink in color, showing occasional traces of bright red. The mycelium of the fungus is readily apparent, and reisolation is easily accomplished from such specimens. Although tuber discoloration is typically vascular, symptoms in pith and cortex have been observed in certain experimental lots, consisting of hard, corky, dark-brown, necrotic areas scattered in the flesh of the tuber (fig. 4).

The first sign of oxysporum wilt under Wisconsin conditions is a yellowing of the lower leaves, which gradually spreads to the leaves directly above. Wilt and death may follow rapidly, or the stem may remain turgid until most of the plant has a golden-yellow cast, with only a few green leaves remaining at the tip. The appearance of the roots, stems, and tubers of infected plants is similar to that described by other workers (*2*, *5*, *8*, *13*, *18*), and these below-ground symptoms are frequently indistinguishable from those of the other fusarium wilts.

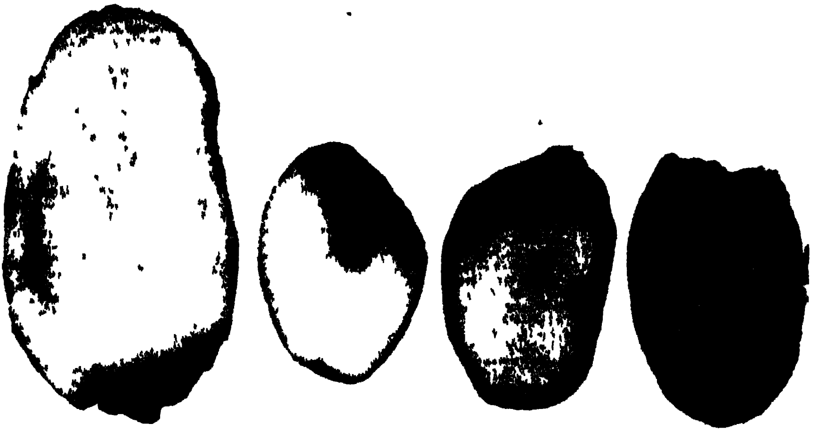

FIGURE 5.—Severely infected tubers from potato plants inoculated with *Fusarium avenaceum* showing vascular discoloration and a dry stem-end rot. Photograph taken at the time of harvest.

The symptoms of eumartii wilt in Wisconsin are similar to those described by Goss (*6*, *8*), and infection is readily secured under a wide range of conditions in the greenhouse or field. Rural New Yorker variety is an exception, frequently showing only the irregular, interveinal bronze areas on the leaflets without interveinal chlorosis. Tip burning and dropping of the leaves occasionally occur independently of any other symptoms. Russet Rural potatoes in commercial fields[5] show this same phenomenon, and similar symptoms have been observed by the senior author on Bliss Triumph potatoes growing in western Nebraska. These plants previously had been retarded by dry weather, so that severe tip burning on the leaves of affected plants was the only apparent sign of the disease on the aboveground parts. Severe seed-piece infection and early death of the plant have been observed only when a large quantity of inoculum was present.

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[5] So far as the writers know, this is the first report of eumartii wilt in Wisconsin.

Leach and Darling[6] in 1935 reported similar symptoms in Minnesota on plants affected by unidentified species of fusaria. In 1939 Leach (*12*) further stated that the wilt caused by the tarnished plant bug was not identical with the purple top wilt, which disease he indicated might be due to the aster yellows virus. Decker[7] reported western aster yellows on potatoes in New York which agreed closely with the descriptions of purple top wilt and the blue stem disease described by Orton and Hill (*14*). Hill (*11*), after examining potato tubers affected with blue stem, purple top wilt, net necrosis, stem end browning, and avenaceum wilt, reported differences in the location of suberized deposits and wound cork, but found no microchemical differences in the wound periderm among the different types of material.

DISTRIBUTION AND ECONOMIC IMPORTANCE OF FUSARIUM WILTS IN WISCONSIN

Avenaceum wilt varies in severity from year to year, and, like oxysporum wilt, is influenced by environmental factors and by the vigor of the host plant. Leaf rolling, reddening of the foliage, and formation of aerial tubers are symptoms which occur chiefly in the northern half of the State, and they appear most prominently after mid-August. For this reason, the late-maturing varieties usually show a higher percentage of infected plants under natural field conditions than do the early varieties. Lower temperatures accompanied by heavy or frequent rains, following a period of drought and high temperatures, commonly lead to the advent of this symptom complex 1 week or more later. In Wisconsin the disease appears to be more prevalent in the light-textured soils.

The resemblance of avenaceum wilt to some other potato disorders and its sporadic appearance make estimation of its economic significance difficult. The amount of infection is generally small, involving from 2 to 5 percent of the plants in a field, although fields showing up to 20 percent infection have been observed, and small areas within a field may show much higher percentages of plants with foliage symptoms than are present in the field as a whole. It is believed, however, that the diseases of *Fusarium oxysporum* and *F. avenaceum* are equal in importance and that these two species are largely responsible for the fungus wilts of potatoes in Wisconsin.

F. solani var. *eumartii* has been isolated from field and bin specimens in this State, and fields showing 8 percent of the plants affected by this organism have been observed. Eumartii wilt, however, is confined largely to the northeast section of the State and even there it is not the serious problem to potato growers that it is in some other parts of the country.

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[6] LEACH, J G, and DARLING, HENRY. SYMPTOMS OF FUSARIUM WILT OF POTATO IN MINNESOTA THIS YEAR U. S Bur Plant Indus, Plant Dis. Rptr 19 299-302 1935. [Mimeographed]

[7] DECKER, PHARES A NEW POTATO DISEASE IN NEW YORK U S Bur. Plant Indus, Plant Dis Rptr. 23 226-227. 1939 [Mimeographed]

RELATION OF THE PARASITE TO THE HOST TISSUE

To observe the path of the fungus in the plant, potato seed pieces were planted in sterilized soil in glass containers, which were covered to exclude light. The soil was moistened with sterilized, distilled water through a tube at one side of the container. A suspension of spores was introduced through the watering tube when the roots had accumulated around the end of the tube. Daily observations were made with a dissecting microscope and plant parts were removed at intervals and prepared for sectioning. *Fusarium avenaceum*, *F. oxysporum*, and *F. solani* var. *eumartii* were studied comparatively in this manner.

Water soaking of the root tips appeared at 2 days with *F. solani* var. *eumartii*, whereas it did not become definite with *F. avenaceum* and *F. oxysporum* until the seventh day. The hyphae of *F. solani* var. *eumartii* were apparent in the cells of the epidermis and cortex of certain rootlets 7 days after inoculation. In the more severely affected rootlets the walls of the cortical cells and of the younger xylem cells were broken down. The epidermal cells were thus separated from the larger cells of the stele but still both groups remained in their original positions. The later stages of invasion with *F. solani* var. *eumartii* were similar to those illustrated by Goss (*6*). The cortex of the older roots became permeated with the intracellular hyphae of the fungus, which eventually reached the xylem. Mycelia occurred abundantly in the cortical cells of the lower stem and were also found in the spiral and pitted vessels at the junction of the infected stem and roots. Some of the smaller pitted vessels of the xylem in the stem collapsed, and the thinner, weaker-appearing cell walls indicated the effect of solvent action; in others, which were still thick-walled, dense granular deposits were found. Necrosis of cell groups in the inner and outer phloem, together with collapse of the xylem, occurred, in advance of the fungus hyphae.

In the early stages of penetration by *F. avenaceum* and *F. oxysporum*, mycelia were found in the epidermal and cortical cells of the young rootlets. The cell walls appeared to have become softened and swollen and some cells were partially separated from the adjoining ones. This dissolving action was not so rapid as that observed with infection by *F. solani* var. *eumartii*. *F. avenaceum* was found commonly in the xylem vessels of roots and stems, and hyphal branches radiated from the infected xylem vessels into the outer phloem of the stem where collapse and necrosis of the cells occurred. Many of the thick-walled xylem cells of the stem became plugged. The cortex of the lower stem was also invaded. In the younger roots infected by *F. oxysporum*, mycelia were found in the xylem vessels of the root, as well as in the cortex. In larger roots and in the lower stem the hyphae occurred chiefly in the xylem.

It may be seen that the three fusaria related themselves to the potato in a very similar manner. *F. solani* var. *eumartii* was the most active cortical invader, and enzymic action in advance of the pathogen was greatest. *F. avenaceum* invaded the cortex of root and stem, but was more aggressive than the last-mentioned fungus as a vascular invader. *F. oxysporum* was about equal to *F. avenaceum* as a vascular parasite, but it was less conspicuous in the stem cortex.

TEMPERATURE AND MOISTURE RELATIONS

In order to determine the effect of natural environment on the production and development of wilt diseases, it is necessary to understand the role of temperature and moisture in infection. Therefore, studies of the effect of temperature on the growth of the avenaceum wilt fungus, together with the effect of soil temperature and moisture on development of the disease, were undertaken.

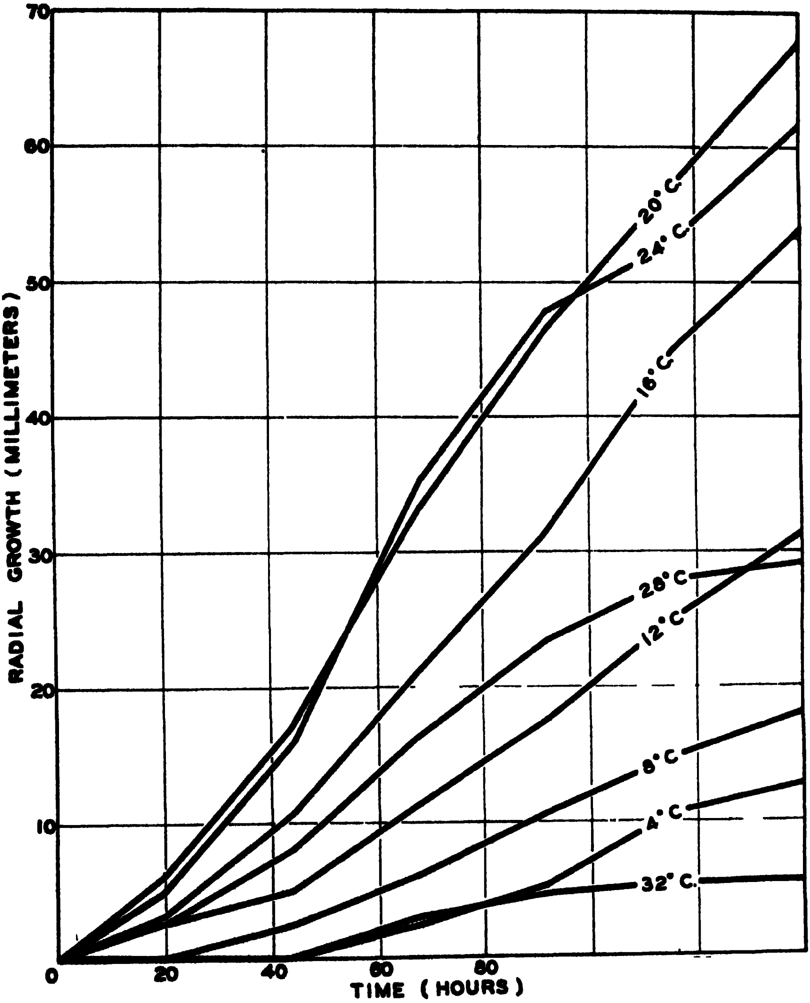

Figure 6.—The effect of temperature on the growth of *Fusarium avenaceum* on potato-dextrose agar.

RELATION OF TEMPERATURE TO GROWTH OF THE FUNGI

Plates of potato-dextrose agar adjusted to neutral reaction were inoculated with 4- mm. disks taken from the periphery of a 5-day-old actively growing Petri-dish culture of *Fusarium avenaceum*. Five replicates were placed at each of eight temperatures ranging at 4° intervals from 4° to 32° C., inclusive. Increments of radial growth were measured daily.

The results are shown graphically in figure 6. Both daily-increment and total-growth measurements exhibited the greatest response at temperatures of 20° and 24° C. These results agree rather closely with those of Cormack (*4*), who reported an optimum of 24° for a strain of *F. avenaceum* isolated from alfalfa. The straight-line trends in this graph indicate that growth was not retarded by the accumulation of staling products or other byproducts of the fungus. The optimum is lower than the optima reported for *F. solani* var. *eumartii* (25°) (*6*) and *F. oxysporum* (30°) (*7*) although Goss (*5*) demonstrated that the optimum temperature for growth of the latter varied with the substrate.

EFFECT OF SOIL MOISTURE AND SOIL TEMPERATURE

Wisconsin soil-temperature tanks were employed in the determination of the effect of soil temperature and moisture on infection and on growth of uninoculated plants. Temperatures were automatically controlled to ±0.5° C. at each of the following temperature levels: 16°, 20°, 24°, 28°, and 32°. The air temperature fluctuated between 20° and 25°. A mixture of one-half sand and one-half composted soil in burlap bags was autoclaved at 20 pounds steam pressure for 2½ hours. Inoculations were made several days before planting, after the soil had been exposed to the air for 10 days. The sand and corn-meal inoculum used in the first series was abandoned in favor of autoclaved barley kernels in later trials. The Katahdin and Irish Cobbler varieties were planted in the first two series, Rural New Yorker in subsequent tests. Four presuberized seed pieces were planted in each can of autoclaved, artificially infested soil. Five separate series were completed. In the first two a comparison was made between plants inoculated with *F. avenaceum*, *F. solani* var. *eumartii*, and *F. oxysporum* and uninoculated controls. In the remaining three series only *F. avenaceum* was used. The soil moisture was maintained throughout at 40 and 60 percent of the moisture-holding capacity of the soil.

The best growth of the foliage of the potato plant was produced in the greenhouse at constant soil temperatures of 20° and 24° C. when the air temperature was maintained at 20° to 25°. As soil temperature increased, germination was hastened and leaf size decreased. At 28° the plants were decidedly smaller, while at 32° growth was slow and abnormal, resulting in poorly developed tops. Plants grown at a soil temperature of 16° developed more slowly than those at 20° and 24°, although thicker stems and larger leaves resulted and the plants ultimately produced more leaf area. Better germination and larger plants prevailed throughout the temperature series at 60 percent of the soil moisture-holding capacity. At 28° and 32° plant growth was retarded at 40 percent moisture, a result undoubtedly of the more rapid depletion of soil moisture by the greater rate of evaporation and transpiration at these temperatures. The lower the temperature, the less was the effect of moisture on plant size.

At the temperatures ranging from 20° to 28° C., the plants in inoculated soil were from 2 to 6 inches higher than the controls while the reverse was true at 32°. At 16° no differences were observed. Root growth in all inoculated plants was correspondingly greater than in the controls. At the lower level of soil moisture a slight increase in the percentage of plants infected by *F. avenaceum* and *F. oxysporum* resulted at 16°, while a decrease occurred with *F. solani*, var. *eumartii* at 28° and with *F. oxysporum* and *F. avenaceum* at 32°, but the number of plants was too small to show a significant difference. The data for the two moisture levels at each temperature are combined in table 2.

TABLE 2.—*The effect of soil temperature on infection of potato plants by three species of Fusarium*

Soil temperature (° C.)	Symptoms in plants in soil inoculated with— *F. solani* var. *eumartii*	*F. oxysporum*	*F. avenaceum*	Symptoms in plants in uninoculated soil
	Percent	*Percent*	*Percent*	*Percent*
16	75	10	12	0
20	100	10	43	0
24	100	70	62	0
28	93	100	100	8
32	(1)	[2] 92	[2] 80	[2] 0

[1] Mechanical difficulties. No infection at time of injury to plants.
[2] Represents 1 series only.

The effect of temperature on *F. oxysporum* and *F. solani* var. *eumartii* was similar to that observed by Goss (*5*, *6*), who reported an optimum of 30° C. for infection by the former and of 20° to 25° by the latter. The average percentage of infection by *F. avenaceum* for the five series at two levels of moisture showed a definite optimum at 28° for each moisture level (table 3), while it was consistently less at the lower moisture level at 16°. However, although the development of avenaceum wilt was more severe as the soil temperature increased up to the optimum, it was also more severe in the drier soil in spite of the fact that the percentage of infection was greater in the moister soil.

Two trials were made with Rural New Yorker to determine the effect of soil moisture on infection when the temperature remained constant. Soil was adjusted to 30 percent, 50 percent, and 70 percent of the moisture-holding capacity. Soil temperature was kept at 24° C. in the first series and at 28° in the second, while the average air temperature was 20° to 25° in both cases. Infection was greatest at the 50-percent level and least at 30-percent in both experiments (table 4). The optimum temperature for growth of *F. avenaceum* is approximately the same as that required for rapid, sturdy development of the potato plant (20°–24° C.) while the optimum soil temperature for infection is some 4° higher. Thus although a difference of more than 5° exists between its growth optimum and that of *F. oxysporum*, they appear to have closely similar optimum temperatures for infection. Infection by *F. avenaceum* was more severe at 50 percent of the water-holding capacity of the soil than at either 30 or 70 percent. Similar behavior was reported for *F. oxysporum* (*5*), while soil moisture has relatively little influence on infection by *F. solani* var. *eumartii* (*6*).

Table 3.—*The effect of soil temperature on infection of potato plants by Fusarium avenaceum at two levels of soil moisture*

Soil temperature (° C)	Symptoms in plants in soil inoculated with *F. avenaceum* at—: 60 percent of moisture-holding capacity [1]	Symptoms in plants in soil inoculated with *F. avenaceum* at—: 40 percent of moisture-holding capacity [1]	Symptoms in plants in uninoculated soil at—: 60 percent of moisture-holding capacity [2]	Symptoms in plants in uninoculated soil at—: 40 percent of moisture-holding capacity [2]
	Percent	*Percent*	*Percent*	*Percent*
16	0	15	0	0
20	50	50	0	5
24	62	58	10	5
28	94	70	5	10
32	72	50	0	0

[1] Average of 5 replicates representing a total of 76 plants.
[2] Average of 5 replicates representing a total of 52 plants.

Table 4.—*The effect of soil moisture on infection of potato plants by Fusarium avenaceum at constant temperature*

Water-holding capacity of the soil (percent)	Symptoms in plants in soil inoculated with *F. avenaceum* [1]	Symptoms in plants in uninoculated soil [2]
	Percent	*Percent*
30	10.3	0
50	53.5	0
70	17.3	3.3

[1] Average of 2 experiments representing a total of 40 plants, 20 at 24° C and 20 at 28° C.
[2] Average of 2 experiments representing a total of 24 plants, 12 at 24° C and 12 at 28° C.

SEASONAL DEVELOPMENT OF THE DISEASE

The expression of symptoms of the fusarium wilts of potato present a constantly changing picture in commercial fields in Wisconsin. In the northern half of the State infected plants display varying stages of rosetting and upward curling of the leaves. The foliage parts are highly pigmented and the buds in the leaf axils are stimulated into the formation of aerial tubers. In the central and southern sections, infection usually results in wilting and rapid collapse of the plants. In still other more or less isolated regions of Wisconsin, the symptoms are very typical of those described for eumartii wilt (*6*). Disease development is undoubtedly influenced by the time and amount of infection and by the soil and climatic environments. The symptoms generally occur late in the growing season. This fact gives the impression that the potato varieties which are normally mature in late August are more resistant than the late varieties. However, Goss (*7*, *8*,) has observed that the amount of infection by *Fusarium* is influenced by date of planting as well as by variety.

In order to obtain a more adequate basis for the interpretation of the development of the wilt diseases throughout the season, the following experiments were carried out. Several varieties of potato were inoculated by various methods and planted on several dates in each of two widely separated locations in the State. These studies were conducted during three successive seasons. Plants affected with avenaceum wilt, oxysporum wilt, and eumartii wilt were compared throughout with the uninoculated plants.

PLAN OF FIELD EXPERIMENTS

In 1937, a factorial experiment was conducted in the field at Madison, Wis., randomized blocks being used. Four potato varieties, three species of *Fusarium*, and three methods of inoculation were tested at each of three dates of planting with the result that potato varieties, fungus species, methods of inoculation, and dates of planting could be studied separately and in combination with each other. One-fourth of the total number of rows in the plot were uninoculated controls. Two standard early, maturing varieties, Irish Cobbler and Bliss Triumph, and two late-maturing varieties, Katahdin and Rural New Yorker, were used. Inoculation was made at the time of planting in all cases. The three methods of inoculation employed, namely, plugged, presuberized, and fresh-cut, have been described in the section dealing with inoculation methods. In several other tests, either corn-meal-sand inoculum or inoculated, sterilized soil was added to the seed piece in a paper bag and then planted. Plantings were made on May 25, June 9, and June 22, in order to include the range of the usual planting season for southern Wisconsin. Notes on the incidence and severity of foliage symptoms were taken at regular intervals throughout the growing season. Tuber discoloration was recorded for each individual lot after harvest on October 5.

The planting plan used in 1938 was similar to that of 1937. The three *Fusarium* species and four potato varieties were again included. Six replicated series were included, all planted on one date.

In order to study more fully the effect of environment on production of symptoms and on subsequent disease development, two locations were used in 1939. In addition to the plot at Madison, a planting was made at Starks, Wis., near Rhinelander, approximately 200 miles north of Madison. Planting in these two locations allowed for the study of the effect of two distinctly different environments on the development of the three wilt diseases. The plot at Madison was set up to study the effect of three methods of inoculation Seed tubers of Irish Cobbler and Rural New Yorker were inoculated in three ways: By dipping freshly cut seed in fungus suspension, by using inoculated soil in paper bags along with the seed pieces, and by employing barley-kernel inoculum in the same manner. Uninoculated and inoculated rows were randomized within each of three replicates. The individual row size was increased to 50 plants for each inoculation as compared with 10 plants used in previous years. The purpose of the larger sample was to obtain greater uniformity and to determine whether any bias had resulted from the smaller test rows formerly employed.

In the plot at Starks six replicates each of Bliss Triumph and Rural New Yorker were planted. Inoculations were made in two ways: By dipping freshly cut tubers in a fungus suspension, and by the use of artificially infested soil. The plot was bordered on one side by grassland and on the other three sides by summer-fallowed ground, and was isolated from any other potato fields by a distance of one-half mile.

All experimental plots were sprayed frequently throughout each growing season with bordeaux mixture and calcium arsenate.

SEASONAL DEVELOPMENT OF SYMPTOMS ON THE FOLIAGE

The rate of development of top symptoms appeared to be closely correlated with the extent of infection, and to be favored by conditions conducive to rapid depletion of soil moisture. Wilt-disease readings were recorded in the inoculated and uninoculated rows as soon as positive symptoms appeared. The percentages of inoculated and uninoculated plants showing symptoms on the foliage at various dates throughout the season for each of 4 potato varieties and 3

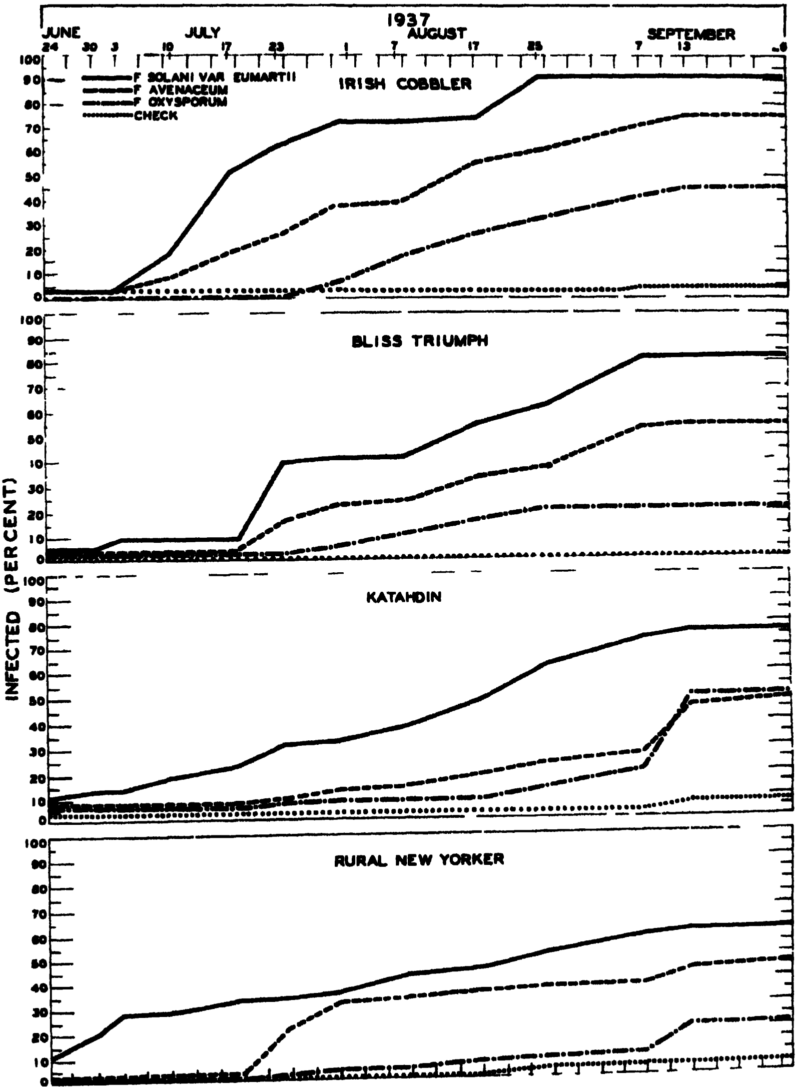

FIGURE 7. The occurrence throughout the growing season of distinctive signs of the disease produced when four varieties of potato were inoculated with each of three wilt fusaria at Madison, Wis.

species of *Fusarium* in 1937, are shown graphically in figure 7. Thus each curve is based on the data from 90 plants. All plants which did not emerge were counted as infected and because of the irregular appearance of symptoms, the results from three dates of planting were combined. The time of appearance of symptoms varied with the individual host plant and with the species of *Fusarium* concerned. Some plants displayed definite signs of the disease soon after emergence, while others remained apparently healthy until the approach of maturity. Inoculation with *F. solani* var. *eumartii* produced the earliest symptoms and consistently showed the greatest percentages of infection, while *F. avenaceum* and *F. oxysporum* maintained a lower level of infection but caused a rapid rise in the number of plants showing symptoms late in the season. The symptoms of *F. avenaceum* were better defined, and therefore the curve for that species is higher than that of *F. oxysporum*. *F. solani* var. *eumartii* is clearly shown to be the most aggressive parasite as measured by both earliness of disease development and by the percentage of plants affected in all varieties. The percentage of diseased plants in the uninoculated rows was uniformly low.

Somewhat lower percentages of affected plants occurred with Bliss Triumph and Katahdin than with Irish Cobbler. These differences are consistent with those discussed later on tuber discoloration in the same varieties. The lower percentages of infection recorded for Rural New Yorker are explained in part by the fact that in this variety, as well as in Russet Rural, symptoms often consist only of tip burning and dropping of the leaves. These symptoms were not distinctive for any individual species of pathogen and were not recorded, therefore, as positive signs of the diseases concerned.

To demonstrate the validity of the graph in figure 7, a measure of the time required for the appearance of symptoms, that is, the average number of days to the appearance of symptoms was calculated, using the time elapsing between planting and the first appearance of symptoms for each plant and giving a value of "zero days" to all nonemerging plants and to plants which died within 1 week after emergence. Healthy plants, i. e., those showing no symptoms before maturity were given a value of 200 days. Low numbers, therefore, indicate early infection and symptom production, while higher numbers approaching 200 show that the plants were healthy throughout the season. If the foliage and tuber symptoms were closely correlated a statistical analysis of foliage symptoms would give results closely comparable to those obtained for the tuber symptoms.

The correlation between the time required for symptom appearance and the amount and severity of tuber symptoms is shown in table 5. A negative correlation ($r_2 = -0.27$) was found between the days required for symptom expression and the percentages of tubers showing symptoms. For the correlation of days and disease index a significant correlation value was obtained ($r_1 = -0.79$). The difference between the two correlations ($Z_2 - Z_1 = 0.79 \pm 0.47$) was significant. These relationships show that days and index values were significantly more comparable than were days and percentage values. In other words, as the time required for primary-symptom expression increased, there was correspondingly less tuber infection and the severity of the latter decreased. It will be noticed that the inverse relationship for days as compared with the amount and degree of tuber infection is maintained in general throughout tables 5, 7, and 8. Analysis of variance of the values for the number of days to symptom expression gave significant differences similar to those found when the data on tuber symptoms were analyzed.

TABLE 5.—*Correlation of the time required for the production of foliage symptoms with the percentage and index of tuber discoloration at Madison, Wis., 1937* [1]

Species of *Fusarium* and potato variety	Average time required for symptom expression	Vascular discoloration of the tubers	
	Days	*Disease index*	*Percent*
F. avenaceum.			
Bliss Triumph	126	3	11
Irish Cobbler	96	10	31
Katahdin	142	5	19
Rural New Yorker	136	17	51
F. solani var. *eumartii*			
Bliss Triumph	85		47
Irish Cobbler	64		70
Katahdin	92		55
Rural New Yorker	108		61
F. oxysporum:			
Bliss Triumph	171		12
Irish Cobbler	144		24
Katahdin	142		19
Rural New Yorker	176		43
Total	1,482	224	443

[1] r_1 of days and index = −0.79 r_2 of days and percent = −0.27 $Z_2 - Z_1 = 0.79 \pm 0.47$ At the 5-percent level of significance, for 10 degrees of freedom, $r = 0.58$

The above correlations (table 5) were derived to ascertain the general association of foliage symptoms with the amount and severity of tuber symptoms. It cannot be concluded that these relationships were always constant, since the type of symptom expressed was influenced by the effect of the species of *Fusarium*, variety of potato, and by several factors of the environment. These variations are discussed elsewhere in the text.

DEVELOPMENT OF SYMPTOMS IN THE TUBER

Vascular discoloration of the tubers from infected plants was used as the criterion for measuring the effect of the various inoculations and treatments, and while it did not always indicate tuber infection, the association of stem infection and foliage symptoms with tuber discoloration was definite. The amount of the latter arising from chance contamination by fusaria or other causes was estimated from the controls which were placed in one row out of every four throughout the plots. The differences between uninoculated and inoculated lots were ascribed, therefore, to the action of the particular species of pathogen used in the inoculation.

The extent of vascular discoloration in each lot of tubers was recorded in four arbitrary grades as follows: Clean—no vascular browning beyond ¼ inch from the point of stolon attachment; slight—discoloration of all vascular elements up to ½ inch, or of several strands up to 1 inch; moderate—all elements discolored ½ to 1 inch from stolon attachment or a few strands farther than 1 inch; severe—greater degrees of infection than the moderate grade. The percentages of tubers showing symptoms were determined for each lot and the indices of vascular discoloration were calculated on the basis of the following class weights: 0—clean, 1—slight, 2—moderate, and 5—severe. There was a definite correlation between the percentage of diseased tubers and the tuber-disease index, but the latter was a better measure of severity of infection of the tuber and it correlated more closely with the foliage-symptom index already presented in table 5.

The influence of the method of inoculation on the development of tuber discoloration as expressed by the disease index is shown in table 6. Among the three methods used in 1937 no appreciable difference was noted with *F. oxysporum*, the fresh-cut method was somewhat better in the case of *F. avenaceum*, while the plugged method was definitely inferior to the other two in the case of *F. solani* var. *eumartii*. This is not in accord with the greenhouse inoculation experiments (table 1), where the fresh-cut method gave consistently a higher tuber-disease index for each species than did the presuberized method. In 1939 the fresh-cut method was compared with methods in which inoculated soil and inoculated barley, respectively, were used. The former of these resulted in the highest tuber-disease index with each species, although in the case of *F. avenaceum* and *F. oxysporum* the differences were not great enough to be significant.

TABLE 6. *The effect of method of inoculation upon development of tuber symptoms as expressed by the tuber-disease index, Madison, Wis., 1937 and 1939*

Method of inoculation	Disease development in tubers from plants inoculated with						Uninoculated	
	F. avenaceum		*F. oxysporum*		*F. solani* var. *eumartii*			
	1937	1939	1937	1939	1937	1939	1937	1939
	Disease index	*Disease index*	*Disease index*	*Disease index*	*Disease index*	*Disease index*	*Disease index*	*Disease index*
Plugged	6.3		7.2		28.9		0.8	
Presuberized	7.8		6.5		44.9		2.1	
Fresh-cut	11.5	9.3	6.3	10.3	48.5	25.9	1.3	3.8
Inoculated-soil		12.9		11.8		41.0		5.9
Inoculated-barley		10.6		10.7		31.7		8.5
Average	8.5	10.9	6.7	10.9	40.8	32.9	1.4	6.1

Goss (*8*) reported that early planting increased tuber infection by fusaria in the field. The difference in infection between planting dates in 1937 was measured by the average number of days required for the appearance of foliage symptoms, by the percentage of diseased tubers, and by the tuber-disease index (table 7). Significantly greater infection was produced by planting inoculated seed-pieces on May 25 than on June 9. No difference in the time required for foliage-symptom production appeared between the planting of June 9 and that of June 22, although significant differences between these dates in percentage and severity of the tuber symptoms did appear. It is apparent that the influence exerted by *F. solani* var. *eumartii* is responsible for the differences obtained between the averages of the first two dates of planting. A marked decrease in the amount and degree of infection with all three pathogens was found at the last planting date.

TABLE 7 —*The effect of three planting dates on symptom production by potato plants inoculated with three different species of Fusarium, as expressed by the average number of days required for appearance of symptoms on the foliage the percentage of diseased tubers and the disease index of tubers Madison, Wis , 1937*

[Each figure represents the data from 120 plants]

Date of inoculation and planting	Inoculated with									Uninoculated			Average		
	F arenaceum			*F solani var eumartii*			*F oxysporum*								
	Days	*Percent*	*Disease index*	*Days*	*Percent*	*Disease index*	*Days*	*Percent*	*Disease index*	*Days*	*Percent*	*Disease index*	*Days*	*Percent*	*Disease index*
May 25	119	33 6	11 5	64	73 3	62 5	138	28 7	9 8	196	5 8	1 1	129 3	35 4	21 2
June 9	134	31 6	8 4	92	60 0	36 0	181	29 1	6 5	191	6 2	1 3	149 5	31 7	13 1
June 22	122	19 6	5 6	106	41 7	23 8	156	16 4	3 8	197	6 0	1 8	145 3	20 9	8 7
Average	125 0	28 1	8 5	87 3	58 3	40 7	158 3	24 7	6 7	194 7	6 0	1 4			
Difference required for significance between dates of planting (19 1)													11 7	4 0	2 5
Difference required for significance between species (19 1													13 5	4 7	2 9

TABLE 8 —*The production of symptoms on four varieties of potato inoculated with three different species of Fusarium, as expressed by the average number of days required for appearance of symptoms on the foliage the percentage of diseased tubers, and disease index of tubers, Madison, Wis , 1937*

[Each figure represents the data from 90 plants]

Potato variety	Inoculated with									Uninoculated			Average		
	F arenaceum			*F solani var eumartii*			*F oxysporum*								
	Days	*Percent*	*Disease index*	*Days*	*Percent*	*Disease index*	*Days*	*Percent*	*Disease index*	*Days*	*Percent*	*Disease index*	*Days*	*Percent*	*Disease index*
Bliss Triumph	126	11 4	2 8	85	47 1	35 9	171	12 4	3 4	198	4 0	2 0	145 0	18 8	11 0
Irish Cobbler	96	31 1	10 2	64	70 1	53 3	144	24 2	7 1	198	6 1	8	125 5	32 9	17 9
Katahdin	142	18 9	5 0	92	54 8	36 3	142	18 9	4 5	189	4 1	9	141 3	24 2	11 7
Rural New Yorker	136	51 1	16 5	106	61 3	37 6	176	43 3	11 7	193	9 8	2 0	153 3	41 4	17 0
Average	125 0	28 1	8 5	87 3	58 3	40 7	158 3	24 7	6 7	194 5	6 0	1 4			
Difference required for significance between varieties (19 1)													13 5	4 7	2 9
Difference required for significance between species (19 1													13 5	4 7	2 9

The differences in tuber discoloration between varieties of potato are shown in table 8. It is apparent that the expression of foliage symptoms (fig. 7) and the amount and severity of tuber symptoms are inversely related. Early foliage symptoms are found on Irish Cobbler; Bliss Triumph and Katahdin varieties showed symptoms later in the season. The atypical foliage symptoms on Rural New Yorker made accurate recording difficult. Two definite levels of tuber infection were found. Irish Cobbler and Rural New Yorker were more severely affected than were the Bliss Triumph and Katahdin varieties. One early-maturing and one late-maturing variety were included in each group. Previous observations under natural field conditions had indicated that the late-maturing varieties were more subject to disease attack, while the early potatoes appeared to be more resistant or escaped the disease. In the light of these results, the slight difference in resistance between varieties is a function of the individual variety and is not dependent on early or late maturity. The tendency of the early varieties to show very little of the fusarium wilt diseases in Wisconsin is probably due to the fact that they escape the period of most favorable environment for expression of symptoms, which normally occurs late in the season. The differences observed between varieties are in agreement with those reported by Goss (7), who found Bliss Triumph to be more resistant to *F. solani* var. *eumartii* under field conditions than was Irish Cobbler.

Notes were taken previous to maturity on the amount and severity of stem and tuber discoloration in individual plants of Bliss Triumph and Irish Cobbler varieties. Tabulation of these data seemed to indicate a relationship between stem and tuber discoloration Correlation of these values on a percentage basis is shown in table 9. Positive correlations were found for Irish Cobbler, $r = +0.75$; for Bliss Triumph, $r = +0.49$; and for the total, $r = +0.63$. The values for Irish Cobbler and the total are significant, but the difference between those for Irish Cobbler and Bliss Triumph is not significant. The percentage and severity of stem infection, as the plants infected with *F. avenaceum* and *F. oxysporum* approach maturity, is commonly greater than tuber infection. With *F. solani* var. *eumartii*, percentages and indices of stem and tuber infection more nearly approach the same level. A definite association of foliage symptoms with stem discoloration exists. Individual stems showing aerial symptoms or wilting toward the end of the growing season were usually found to show discoloration of the vascular elements in the below-ground portion of the stem. From the data presented above, one may assume that vascular discoloration of the tubers is an adequate measure of infection where statistically significant differences are obtained following field inoculations.

The data discussed so far with reference to the occurrence of tuber symptoms in field experiments were collected in 1937 and 1939. Those collected in 1938 were not included because a severe epidemic of late-blight tuber-rot in the experimental plot made the analysis of results difficult. A tabulation of the rotting observed in various lots showed the differences in results between varieties of potato but not between the fungus species and controls. For this reason, Rural New Yorker was not included in the analysis, and Irish Cobbler, Bliss Triumph, and Katahdin were treated separately.

TABLE 9.—*Correlation of the percentages of stem and tuber infection on two varieties of potato* [1]

[Each figure represents the data from 30 plants]

Species of pathogen and method of inoculation	Irish Cobbler		Bliss Triumph	
	Vascular discoloration		Vascular discoloration	
	In stems—	In tubers—	In stems—	In tubers—
	Percent	*Percent*	*Percent*	*Percent*
F. oxysporum:				
Fresh-cut	83	22	15	30
Presuberized	52	35	37	17
Plugged	59	43	25	8
F. solani var. *eumartii*:				
Fresh-cut	100	82	87	89
Presuberized	94	80	86	66
Plugged	100	77	79	39
F. avenaceum:				
Fresh-cut	94	59	87	28
Presuberized	83	50	93	18
Plugged	65	27	58	7
Total [2]	730	475	567	302

[1] r_1 for Cobbler = +0.75, r_2 for Triumph = +0.49, r_3 for total = +0.63. At the 5-percent level of significance for 16° of freedom, r = .47; 7 of freedom, r = .67.

[2] Grand total for vascular discoloration in stems, 1,297, in tubers, 777.

Statistical calculations on the percentage and index of tuber infection showed that *F. solani* var. *eumartii* produced significantly more infection than did the other species, whereas there was no significant difference between avenaceum wilt, oxysporum wilt, and the controls. Since it was possible that the variations caused by late blight rot were masking the differences, a square-root transformation analysis was calculated, in which the square root of the percentage value for each lot was extracted and an analysis was computed from these values (table 10). No attempt will be made to prove the validity of such calculations on these data, but transformation analyses have been successfully used to demonstrate the significance of small differences occurring between samples when these differences were overshadowed by other samples with higher values (*3*). *F. solani* var. *eumartii* again showed significant differences in infection throughout. When transformation values were compared, the percentages of discolored tubers from Katahdin plants inoculated with *F. oxysporum* and from Irish Cobbler plants inoculated with *F. avenaceum* were significantly greater than those from uninoculated plots.

TABLE 10.—*The averages for the percentages and square-root transformation of vascular discoloration produced by the three species of Fusarium on three varieties of potato at Madison, Wis., 1938* [1]

[Each figure represents the data from 120 plants]

Potato variety	Inoculated with						Uninoculated		Average	
	F. avenaceum		*F. solani* var. *eumartii*		*F. oxysporum*					
	$\sqrt{N}$	*Percent*	$\sqrt{N}$	*Percent*	$\sqrt{N}$	*Percent*	$\sqrt{N}$	*Percent*	$\sqrt{N}$	*Percent*
Bliss Triumph	3.32	14.2	7.84	63.0	4.27	18.5	3.49	12.8	4.73	27.1
Irish Cobbler	3.90	15.3	8.08	65.8	3.52	13.2	1.97	8.0	4.37	25.6
Katahdin	2.68	9.2	4.88	30.8	3.79	13.5	1.50	4.7	3.21	14.5
Average	3.30	12.9	6.93	53.2	3.86	15.1	2.32	8.5		-

[1] The differences required for significance between *Fusarium* species and controls at the 5 percent level for percentages in Bliss Triumph is 13.8, Irish Cobbler 11.1, and Katahdin 18.0, for $\sqrt{N}$, 1.0 between *Fusarium* species and controls, 0.9 between varieties and 1.7 (variety×species) for comparing species with each other or with the control within a variety.

RELATION OF ENVIRONMENT TO DISEASE DEVELOPMENT IN THE FIELD

Soil and air temperatures and rainfall were studied in relation to the seasonal development of the three diseases. In 1937, the daily mean air temperature was at or above 24° C. for a period of 38 days, July 11 to August 17 inclusive, and the daily mean soil temperature for the same period was at or above 26°. There was a close correlation between soil and air temperature.

A direct relationship appeared to exist in which temperature, soil moisture, and the amount of infection directly influenced the rate of increase in the severity of symptoms. The effect of environment, while one of predisposition to infection, could not be measured on noninfected plants, and since infection did not occur on all inoculated plants, there seemed to be an element of chance involved in infection and the degree of disease development in the individual plant. The symptoms on one plant, however, ultimately reached the same level of severity as that on any other plant affected by the same disease It was in this changing picture, rather than in the percentage of infection, that the effect of environment was most apparent.

Low soil moisture, together with high soil and air temperature following infection, increased the severity of disease symptoms and resulted in the rapid collapse of the individual diseased plant. The intervention of a light rain, even though the temperature remained high, extended the period between the first visual symptom and death. These phenomena may be influenced by the rate of transpiration of the plant and the amount of available moisture.

Soil and air temperatures were low in 1938, and periods of rain and cloudy weather were more frequent and more evenly distributed than in 1937. The foliage symptoms of the oxysporum and avenaceum wilts were not distinct. Eumartii wilt, on the other hand, displayed early definite symptoms, and continuous symptom expression occurred throughout the season, although the number of infected plants in 1938 was not so great as in 1937.

Foliage-symptom development at Madison in 1939 was intermediate between that found in the 2 previous years. *F. solani* var. *eumartii* consistently produced symptoms of interveinal chlorosis of the apical leaves, followed closely by leaf-burning and dropping of the lower leaves. Plants affected by *F. avenaceum* and *F. oxysporum* showed only severe leaf-burning in the more advanced stages of the respective diseases. These effects were not generally discernible from drought or leafhopper injury and no definite differences in symptoms could be detected between the two fungus species. The effects, however, were decidedly more pronounced on the inoculated plants than on the control plants. The progression of disease on the individual plant was apparent, as in former years, and resulted in premature death.

In the study of the three inoculation methods, the earliest expression and most rapid increase in severity of foliage symptoms were observed where the barley-kernel inoculum was used. The infected plant was killed in a shorter time after the presence of the disease was indicated. Infection from inoculated soil was not as rapid nor as severe while the dip inoculation produced the mildest type of infection as measured by foliage symptoms.

The temperature at Rhinelander in 1939 was consistently lower than at Madison when the mean daily temperatures of the two locations are compared (fig. 8). The daily minimum, or night temperature, at Rhinelander was generally 8° to 10° C lower than at Madison, while the daily maximum, or day temperature, was similar in the two locations. These facts accounted for the similarity in the trends of

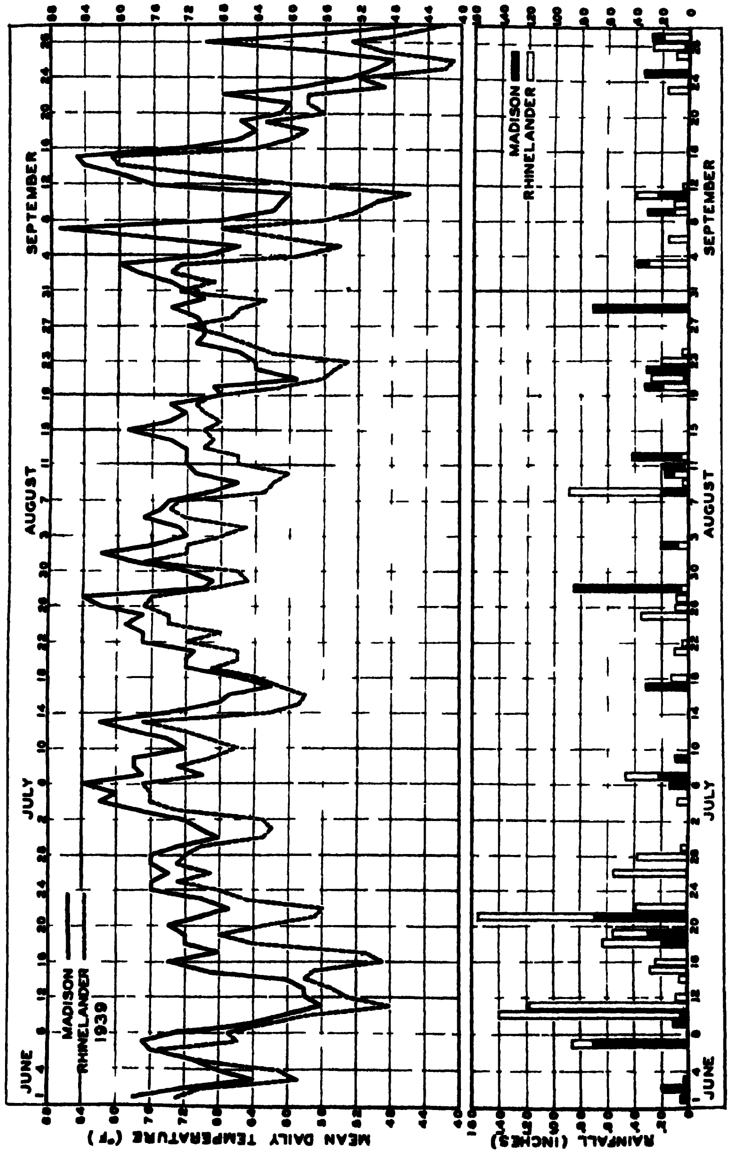

FIGURE 8.—The rainfall and mean daily temperature at Rhinelander and Madison, Wis., for the 1939 growing season.

mean daily temperatures for the two environs, and the lower daily means at Rhinelander It is possible that the lower mean temperature and the greater daily fluctuation found at Rhinelander were largely responsible for the different symptoms produced in the northern plot.

The infected plants at the northern location showed varying symptoms of yellowing and burning of the leaves throughout July and early August. When notes were taken on August 12, however, there was a marked change in symptom expression. Numerous infected plants of both Bliss Triumph and Rural New Yorker varieties had taken on the characteristic purple-top appearance. There was an upward rolling of the leaf-margins, accompanied by a harsh, almost leathery texture of the leaves. The axillary buds were stimulated into the formation of aerial tubers (figs. 2, 3) which were deep red to purple in color. The adjacent stems and petioles were abnormally thickened and frequently carried traces of the red or purple coloration, as did the apical leaves of infected plants. Aerial tubers were not produced by infected plants at Madison in 1939 nor in previous years.

The 2 weeks preceding August 12 at Rhinelander were characterized by several showers, ending a period of relatively high temperature and low moisture (fig. 8). The average temperature at this time showed a downward trend which was a result of the lower night temperatures. It is possible that the high soil moisture accompanied by cooler temperature is responsible for the production of purple-top symptoms on the plants. Similar symptoms appeared in commercial potato fields of this region at the same time.

The differences between the *Fusarium* species in their symptom production at Starks, in the northern part of Wisconsin, are seen in table 11. Eumartii wilt produced the most severe leaf burning and wilt, while the yellowing and tip burning in plants affected by avenaceum wilt and by oxysporum wilt were greater than in the control plants. A small number of plants infected with *F. solani* var. *eumartii* and *F. oxysporum* produced purple-top symptoms, and a limited number of such plants were found in the control rows. Avenaceum wilt produced the greatest number of plants exhibiting this symptom. All such plants evidenced fusarium infection of roots and stems, from which the respective organism was readily reisolated. In a similar plot at Madison, in the southern part of the State, no symptoms of purple top were produced.

TABLE 11.—*The production of foliage and tuber symptoms on 2 varieties of potato by 3 species of Fusarium at Starks, Wis, 1939*

[Each figure represents 150 plants]

Species of *Fusarium* and variety potato	Plants showing foliage symptoms				Tuber symptoms	
	Wilt or severe leaf burning		Purple-top symptoms			
	Aug 12	Sept 3	Aug 12	Sept 3		
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Disease index*
Bliss Triumph						
F solani var *eumartii*	82.5		4.4		54.5	24.7
F avenaceum	63.0		28.6		23.7	10.5
F oxysporum	88.0		11.8		22.2	11.6
Uninoculated controls	24.6		3.6		4.5	1.5
Rural New Yorker						
F solani var. *eumartii*	79.7	100.0	9.3	7.2	52.1	28.6
F avenaceum	81.9	85.0	43.2	48.0	29.9	17.2
F oxysporum	24.0	100.0	5.8	5.8	20.9	8.1
Uninoculated controls	12.0	37.0	3.6	7	4.9	1.5

The plants showing purple-top symptoms sometimes evidenced a tendency to set underground tubers close to the stalk of the plant. In other such plants, normal tuber setting was found. Tubers from the infected plants displayed vascular discoloration and infection similar to those found in the plot at Madison and with natural infection in the field. The relation between the three fungus species in their ability to cause tuber infection at the Starks plot was similar to that found at Madison in 1939 and in previous years.

DISCUSSION

Examination of the literature discloses many potato disorders which produce similar macrosopic and microscopic effects on the host plant. In a review of leaf roll and associated troubles, Orton in 1914 (*15, p. 19*) stated, "The literature on leaf-roll has become so voluminous that few will undertake to peruse all the contributions, which are, indeed, of very uneven merit, and anyone who attempts it is likely to emerge with his concepts of the disease more confused and hazy than at the start." Since that time the symptoms and modes of transmission of the virus disease known as leaf roll have been defined more fully and the distinction of this disease from others with which it has symptoms very much in common has been more fully recognized. The history of the term "Blattrollkrankheit" has been ably reviewed by Pethybridge (*16*). In recent years, psyllid yellows has been distinguished as a member of the same symptom group, while another, the ring rot disease [*Phytomonas sepedonica* (S. & K.) Bergey et al.] is receiving an increasing amount of attention in this country.

Since a common host reaction is displayed when any one of a number of stimuli is present in a favorable environment, it is not surprising that quite similar symptoms are reported in association with many unrelated causal entities. It is probable that other weak parasites, possibly other species of *Fusarium* not yet described on potato, are capable of causing similar host reactions when conditions of environment favorable for infection are followed by temperatures and soil-moisture conditions which promote the expression of such symptoms. The disease with which *F. avenaceum* is associated is similar in its symptoms to several other diseases but it also differs from them in certain respects.

The foliage symptoms produced by the wilt diseases served as one method of measuring infection. Increase in severity appeared to be a function of the relation between the amount of infection in a plant and climatic conditions which hastened the depletion of soil moisture. A visible association between foliage symptoms and the amount of infection within the stem and roots appeared. A relationship also existed between the amount of stem infection and the amount of vascular discoloration in the tubers.

The time of appearance of foliage symptoms was also used as a measure of disease incidence. The average time which elapsed between inoculation and the expression of symptoms was treated statistically and was found to express the same relationships between various tests as did the data obtained from records on the vascular discoloration of the tubers. Vascular discoloration of tubers as expressed by the tuber-disease index appeared to be a reliable method of expressing the differences between the rates of disease development in the test treatments.

Goss (*5*, *6*, *8*) has emphasized the fact that *F. solani* var. *eumartii* is a more aggressive parasite on potato than is *F. oxysporum*, and he states clearly the difference between the pathogenicity of these two species. In the field experiments reported in this paper these species together with *F. avenaceum* have received identical treatment in the experiments upon methods of inoculation, dates of planting, and the host varieties tested. Disease records were taken at the same time and in the same manner, and the inoculated plants in any one series were subjected to identical conditions of environment throughout. The individual inoculations were randomized within replicated blocks to minimize any bias and to reduce the error involved in the accumulation of data.

The effect of the pathogenic species on the production of potato wilt and tuber discoloration is seen throughout the tabular presentation of the results of three seasons' work (tables 5 to 11, inclusive). Inoculation with any one species has resulted in a greater amount of vascular discoloration of stems and tubers than was found in the uninoculated plants. The small amount of natural infection in the control plants did not interfere with the statistical treatment of the data nor with the comparison of the three species of wilt pathogens The relation between the diseases caused by the species of *Fusarium* and the control plants was the same when measured by 50-plant samples in 1939 as by 10-plant samples of the two previous years.

Inoculation with *F. solani* var. *eumartii* resulted in the early appearance of foliage symptoms, a high rate of plant mortality, and a high percentage of severely infected tubers. The percentages and severity of foliage and tuber symptoms were consistently greater, and were frequently double that exhibited by plants inoculated with *F. oxysporum* or *F. avenaceum*. Eumartii wilt was influenced most by different dates of planting, by different methods of inoculation, and by the varieties of the host plant used, although similar trends were recorded for avenaceum wilt and oxysporum wilt. *F. solani* var. *eumartii* is capable of attacking the host plant under conditions of moisture and temperature which are optimum for the development of the host.

F. oxysporum was a less aggressive parasite than *F. solani* var. *eumartii* and the effects of oxysporum wilt were apparent later in the growing season. In this respect and in other ways, *F. avenaceum* and *F. oxysporum* were similar. Both avenaceum wilt and oxysporum wilt are late-season diseases in Wisconsin, and both produce the greatest wilting effect on the host plants either during or following a period of hot, dry weather. *F. avenaceum* and *F. oxysporum* were similar in infectivity when the diseases were measured by foliage symptoms or by the percentage and severity of discoloration in the tubers. No significant statistical differences were found between the disease effects produced by these two fungi. Infection in the control plants was consistently lower and infection by *F. solani* var. *eumartii* was always higher than that produced by either avenaceum wilt or oxysporum wilt.

The similarity of behavior of *F. avenaceum* and *F. oxysporum* observed throughout this series of experiments is of interest when the growth-temperature relationships of the two fungi are considered. A difference of 6° to 8° C. was found for the optimum growth of the two species on artificial media. However, the conditions of temperature and moisture which favored infection and disease development were similar for the two pathogens.

Differences found between inoculation methods could generally be correlated with the abundance of fungus inoculum near the roots of the potato plant. The effect of allowing the seed pieces to heal before inoculation was not as great as was to be expected and did not influence the percentage of diseased tubers. The severity of disease in the tubers was greatest and foliage symptoms appeared earliest when seed pieces were inoculated by dipping in a spore suspension or when inoculated soil or inoculated barley kernels were placed in proximity to the seed piece at the time of planting.

Early death of the plant due to seed-piece infection by wilt fusaria was not found commonly under Wisconsin conditions. This effect was observed only when a large amount of inoculum was placed in contact with the seed piece. Favorable soil moisture and temperature at planting time probably aid in the formation of wound cork and allow the plant to become established to the extent that seed-piece infection does not result in early death.

The effect of different planting dates on the incidence of the wilt diseases may be partially accounted for by the favorable influence of the moist soil early in the season. Cool soil temperatures were especially favorable to infection by *F. solani* var. *eumartii* at the early planting date, while soil moisture had decreased and soil temperatures had increased at the later dates of planting.

The differences between potato varieties in the amount of wilt observed is in agreement with previous reports in the literature. These differences may vary, however, with the environment, since changes in date of planting and harvest, recording the disease data, and other factors may influence differently the level of infection determined for each variety.

Leaf rolling, reddening and rosetting of the foliage, and production of axillary aerial tubers are common with avenaceum wilt in northern Wisconsin. These effects are not usually observed in the southern part of the State. Cool night temperatures or a fluctuating daily temperature appear to play some part in the production of these purple-top symptoms when the soil moisture is sufficient to prevent rapid desiccation and wilting of the infected plant.

SUMMARY

Numerous diseases of potato have been described, the symptoms of which exhibit striking similarity to the various stages of the wilt diseases caused by *Fusarium avenaceum*. The symptoms of avenaceum wilt are compared with those of other diseases, including eumartii wilt and oxysporum wilt. *F. avenaceum* is compared with both *F. oxysporum* and *F. solani* var. *eumartii* throughout the paper.

A strain of *F. avenaceum* isolated from diseased potato plants was capable of infecting and producing vascular discoloration of the roots, stems, and tubers of inoculated plants under both greenhouse and field conditions. Several single-spore lines of this culture differed slightly from each other in their attack on the host plant.

Penetration of the roots occurred rapidly when inoculated with each of the three pathogenic species. Hyphae of each of the fungi were found in the roots and lower stem infected plants. The mycelia of *F. oxysporum* were closely confined to the xylem vessels of the stem; those of *F. avenaceum* occurred abundantly in both the vascular and cortical tissues of the lower stem, while the mycelia of *F. solani* var. *eumartii* were most abundant in the stem cortex. Heavy-walled xylem cells filled with a dense granular deposit and disintegration of certain cells of the phloem and xylem of the stem were found to be associated with infection by each of the three pathogens. Abnormal effects in the host tissues in advance of fungal invasion were greatest in plants inoculated with *F. solani* var. *eumartii.*

The air temperatures which favored optimum growth of potato and of the strain of *F. avenaceum* used were found to be between 20° and 24° C. A higher soil temperature (28°) was most favorable for infection of the potato plant. A moist condition of the soil, with the water-holding capacity maintained at 50 per cent, was more conducive to infection than were either dry or wet soils with the moisture capacity at 30 or 70 percent, respectively.

Inoculation of the seed pieces at planting time was used successfully to induce development of the three wilt diseases in the field experiments. The effects of various treatments were measured by the time required for foliage symptoms to appear and the severity of tuber symptoms. These two criteria of infection were treated statistically. Differences occurring between methods of inoculation appeared to be closely correlated with the amount of inoculum in proximity to the plant.

Irish Cobbler and Rural New Yorker varieties were more susceptible to attack by the wilt fungi than either Bliss Triumph or Katahdin

Early planting of inoculated seed pieces resulted in a greater percentage of infection and in more severely diseased tubers than did late planting.

Eumartii wilt was the most aggressive and resulted in the greatest percentage of severely infected plants. *F. avenaceum* was similar to *F. oxysporum* in the percentages of infection, the severity of disease, the time of appearance of foliage symptoms, and the temperature most favorable for infection. The two pathogens differed slightly from each other in their temperature requirements for growth and the symptoms produced on infected plants.

Infected plants at Starks, in the northern part of the State, displayed leaf rolling, reddening and rosetting of the foliage, and aerial tubers in the axils of the leaves. This type of symptom was not pronounced in a similar plot at Madison in southern Wisconsin. The greatest percentage of plants showing purple top resulted from inoculation with *F. avenaceum*, some were found associated with oxysporum wilt, but few with eumartii wilt.

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MICROBIAL RESPONSES TO ORGANIC AMENDMENTS IN HOUSTON BLACK CLAY[1]

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INTRODUCTION

King (*5*)[3] and various colleagues in a series of papers reported the control of cotton root rot (*Phymatotrichum omnivorum* (Shear) Dug.) by the application of large amounts of stable manure to irrigated plots of alkaline soil in Arizona. Preliminary results, especially those obtained by the use of Cholodony slides (*6*), pointed to great increases in the microflora of the soil as accompanying, perhaps causing, the reduction in growth and activity of the root rot fungus. Since the same disease has produced losses in the cotton crop running into millions of dollars each year for over half a century in Texas (*3*), study of soil microbiological aspects of the problem under Texas conditions was necessary.

The soils infested with the root rot organism in Texas belong to the Houston series and related soil types. These soils constitute the black land prairie areas of Texas; they are alkaline in reaction, ranging from about pH 8 to 8.5 or even 8.7. They form a group of highly colloidal and close-textured calcareous types, with high moisture-holding capacity and with a tendency to become very hard and to crack in dry periods. Thus they present a pronounced contrast to the northern and eastern soils, which have formed the basis for most studies of the microbial floras of American soils.

Microbiological studies of Houston soils, exclusive of those dealing with the distribution of the *Phymatotrichum* fungus, have been limited. Werkenthin (*14*) isolated and identified some of the saprophytic molds of the Austin region; Morrow et al. (*8*) isolated and identified the mold flora of another area; Williams (*15*) noted some bacterial contents of virgin and cultivated soils; and Lewis (*7*) determined the distribution of green fluorescent bacteria in several Texas soils, including Houston clay. As a background for planning further experimentation with these difficult soils, a comparative study of microbial floras under environmental conditions presented by certain selected plots in cotton fields is reported here.

EXPERIMENTAL PROCEDURE

Field-soil samples were taken periodically from November 11, 1937 to December 15, 1938, from plots under different types of crop man-

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[2] In this work the senior author acknowledges the assistance and counsel of H. V. Jordan, of the Bureau of Plant Industry, and Professors I. M. Lewis, G. W. Goldsmith, and Mario B. Morrow, of the University of Texas.

[3] Italic numbers in parentheses refer to Literature Cited, p. 533.

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agement. Two of the three plots chosen for study were from an experiment designed for comparing the effect of turning under a second crop of sorghum with the effect of continuous cotton cultivation upon the incidence of root rot of cotton. These plots were located in Soil Fertility's[4] experiment No. 12 on Blank's plantation, near Lockhart, Tex. Sorghum residues, turned under on October 25, 1937, were estimated at about 3,000 pounds, dry weight, per acre. A third plot was chosen from another Soil Fertility experiment on the Voelker farm near Kimbro, Tex., on which cowpeas were grown in 1937 and the entire crop turned under on October 18, 1937. The area carrying cowpeas had not been selected for this experiment at the time the crop was turned, so the amount of material turned is not known. The growth was good. All three plots were cropped to cotton in 1938.

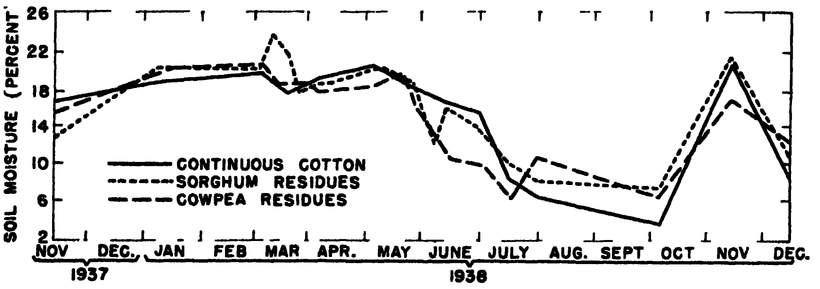

FIGURE 1.—Soil moisture, as determined by air-drying, for the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments.

Soil samples were collected from approximately 4-inch and 12-inch levels; each soil sample was a thoroughly mixed composite of roughly equivalent soil portions taken from four separate locations. Soil and air temperatures were taken at the time of collection of field samples. Cultures were made from moist soils as soon after collection as practicable. The total number of micro-organisms was determined from Petri-dish cultures on sodium albuminate agar; the number of actinomycetes on glycerol nitrate-soil extract agar; and the number of filamentous fungi on acid-dextrose-peptone agar. Spores of aerobic bacilli were determined by plating out the appropriate dilutions on nutrient beef agar after pasteurization at 80° C. for 10 minutes. Quantitative estimates of blue-green fluorescent bacteria were made by the dilution-tube method, on an asparagine medium, and the most probable numbers were determined from the tables of Halvorson and Ziegler (*4*). Soil moisture (fig. 1) was determined by air-drying 100-gm. portions of moist soil; the observed loss in weight was expressed as percentage of moisture in moist soil.

RESULTS

Population changes for various microbial groups found in Houston black clay at different seasonal dates and under different conditions of soil management are presented graphically in figures 2 to 6 for the

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[4] Former Division of Soil Fertility Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

4-inch level of sampling. The results obtained from samples taken at the 12-inch level followed the same general course. The numbers of micro-organisms were conspicuously lower than those for the upper level. Although the figures were obtained and tabulated they are omitted here as not changing the significance of the studies made at the 4-inch level.

TOTAL PLATE COUNTS

The period of maximal activity for all plots, as indicated by the total counts (fig. 2), was found to begin in March and to end early in June. The greatest activity was shown in soil receiving organic residues. The highest total counts in the plots receiving sorghum residues were 428.4 million per gram of dry soil, and for plots receiving cowpeas, 430.7 million, whereas the largest population observed in the continuous-cotton soil was 179.4 million.

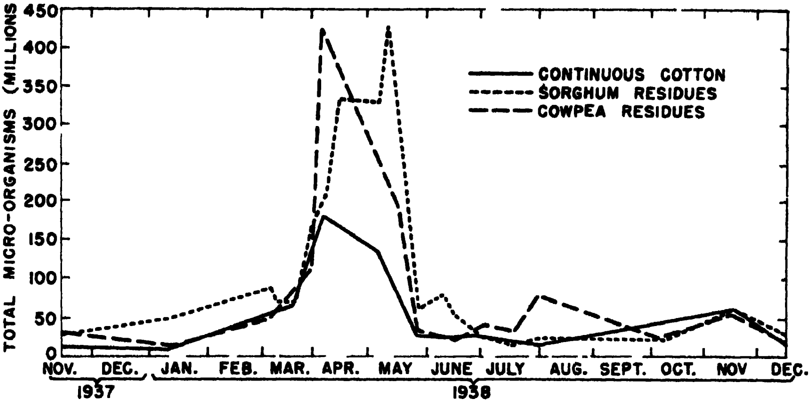

FIGURE 2. - Total counts of micro-organisms in the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments.

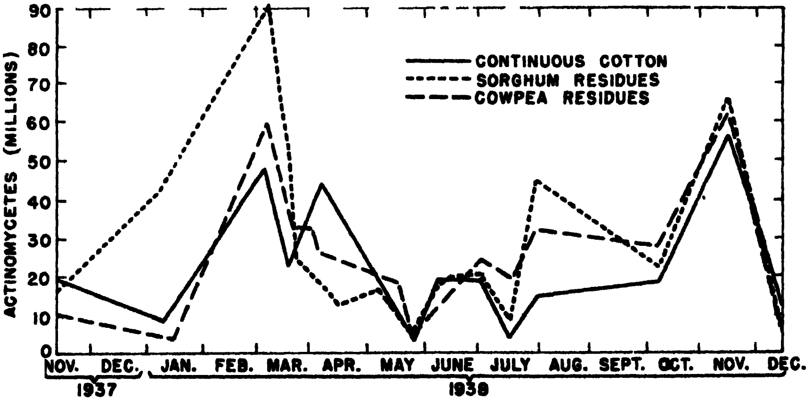

FIGURE 3.—Counts of actinomycetes in the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments.

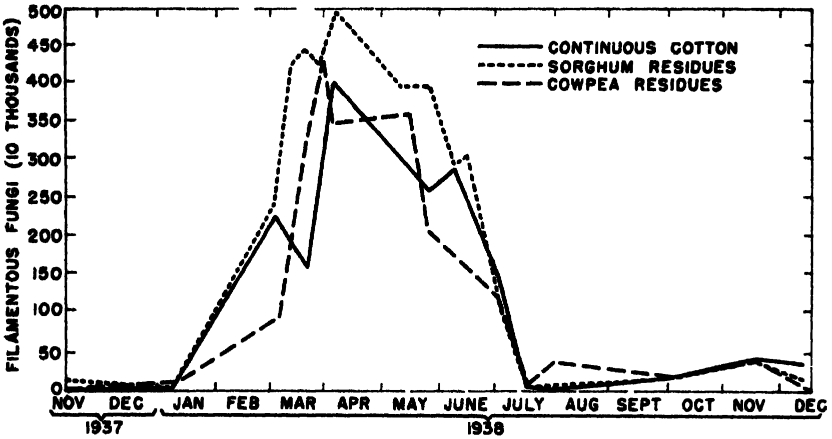

Figure 4.—Counts of filamentous fungi in the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments

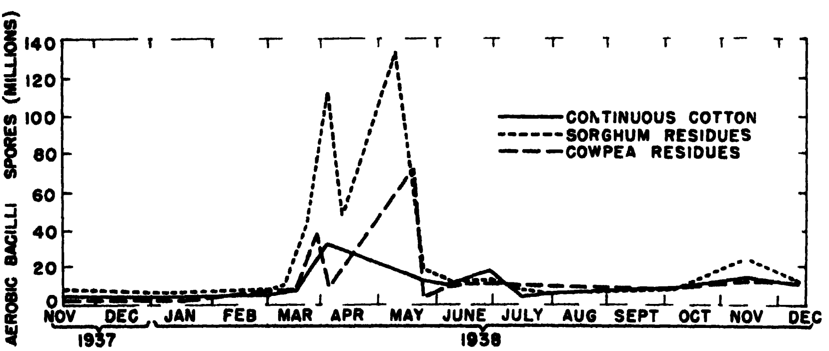

Figure 5.—Counts of aerobic bacillus spores in the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments.

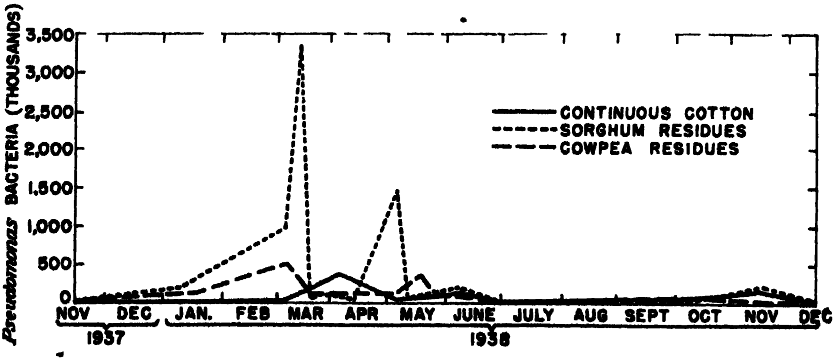

Figure 6.—Counts of *Pseudomonas* bacteria in the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments

The numbers of micro-organisms found in these samples of Houston black clay are greater than those generally reported in studies of soil. Waksman (*13*) has reported from 7 to 10 million total bacteria per gram for rich garden and cultivated orchard soils in New Jersey; Erdman's data (*2*) on Iowa Carrington loam reveal generally 7 to 15 millions per gram; and Vandecaveye (*12*) reports from 3 to 20 millions per gram in his studies on Palouse silt loams in Washington.

FUNGI

The fungi, like the bacteria, showed extensive increases during the spring months. This increase in fungus population became apparent earlier in the season and continued later into the summer than did that of other microbial populations (figs. 2 and 4). The number of fungi in the three soil treatments studied showed smaller differences according to type of soil management than did those of other groups of micro-organisms considered.

ACTINOMYCETES

Increased numbers of actinomycetes appeared during the late winter period in all plots (fig. 3). In early March, the colony count per gram of soil reached 47.5 million for the continuous-cotton soil, 89.5 million where sorghum residues were added, and 59.7 million where the cowpeas were plowed under. The fluctuations shown in colony count of actinomycetes have not been explained, but the totals involved are strikingly greater than those shown in previously reported studies (*2, 13*).

In a subsequent experiment at the Greenville station, sorghum buried deeply in bundles over which cotton was planted was dug out in the last week of August 1939. As the area was broken open the odor of *Actinomyces* was detected strongly, and sorghum stalks only partly decomposed were powdery white with *Actinomyces* spores. In the buried bundles, the presence of open spaces or cracks was an obvious factor in the development of powdery spore masses. Temperatures in the soil, 10° to 20° higher than those found in northern and eastern soils, may be an important factor in the totals found.

SPORES OF BACILLI

Changes in the number of spores of aerobic bacilli (fig. 5), under different seasonal conditions and under different types of soil management, parallel roughly the changes observed for total microbial populations. Maxima were obtained during the spring period; thus the effect of organic residues was apparent. The maxima for unamended soil and for treatments with sorghum and cowpea residues were 31.4 millions, 132.4 millions, and 72.5 millions, respectively.

The blue-green fluorescent bacteria (fig. 6) showed response to added organic residues during the latter part of the winter. Maxima observed for the sorghum- and the cowpea-residue amended soil were 3,400 thousands and 500 thousands, respectively, and for the unamended soil, 370 thousands. The first two values were observed in early March, and the last in early April. Like total bacteria and sporulating bacteria, *Pseudomonas* types showed depressed numbers during the summer and early fall months.

DISCUSSION AND INTERPRETATION OF RESULTS

Study of figure 7, showing the general temperature relations through 1 calendar year, shows that soil temperatures at the 4-inch level fell below 60° F. about December 1, 1937, and again reached 60° early in February, and 70° by March 1, 1938. At no time were freezing temperatures observed. Nevertheless, the cooler temperature shown would readily account for the fact that the total number of micro-organisms was static (fig. 2) from the latter part of November to the early part of March. Very low moisture content in the latter part of July to October clearly correlates with low bacterial activity during that period. Such observations are in entire harmony with the observations of many workers (*1*, *2*, *4*, *9*, *13*).

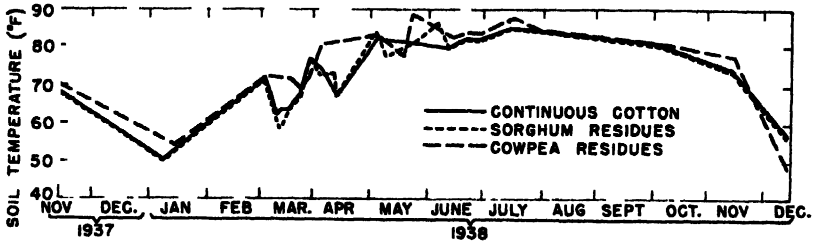

FIGURE 7.—Soil-temperature readings for the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments.

Comparison of figures 1, 2, and 7 shows a rather sharp fall of temperatures beginning about November 1, 1937, accompanied by small changes in bacterial population, as indicated by the colony count in Petri-dish cultures. Although there was a differential rise in number of actinomycetes during the winter months (fig. 3), the total flora as shown by colony count (fig. 2) changed little before the temperature rose to 70° F. again early in March. The abrupt maxima reached in April and May can therefore be interpreted as the resumption of bacterial decomposition in the organic matter plowed under late in October. This activity would appear to have been arrested by the falling temperatures. The peak number for the 4 spring months, reaching more than 400 million micro-organisms, represents the normal rise where such amounts of plant materials are plowed under (compare Smith and Humfeld, *10*, *11*). It is noteworthy that in the plot receiving the residues from the previous cotton crop alone, the rise was simultaneous, even though the highest figure was somewhat under half (179 millions) of that for the green crops plowed under.

Transferred to Texas conditions, King's hypothesis that the introduction of masses of organic matter would produce great microbial activity is fully confirmed. The numbers reported here from colony counts in Petri-dish cultures are strikingly higher even than those of Smith and Humfeld (*10*, *11*) from their composite samples.

SUMMARY

Curves for temperature, moisture, and microbial numbers were established for selected plots of Houston soil as a background for studies in root rot control. As an environment for microbial activity, the plots of Houston soil studied furnish the following striking features:

(1) In a period between early December and February the temperatures remained below those required for active microbial multiplication, yet without freezing.

(2) Temperatures ranged above 70° F. from March to November, and from 80° to 90° from June to October.

(3) Bacteria and actinomycetes are much more active and abundant than has been reported for northern soils. Maxima for total colony counts in soils receiving organic amendments reach 200 to 400 millions; actinomycetes at times reach 50 and 90 millions to the gram. Such responses to added organic nutrients present a challenge to the worker to search for organic media and agronomic practices capable of yielding a controlled microflora.

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SOIL BACTERIOLOGICAL STUDIES ON THE CONTROL OF THE PHYMATOTRICHUM ROOT ROT OF COTTON[1]

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INTRODUCTION

Search for methods of control or elimination of the cotton root rot fungus (*Phymatotrichum omnivorum* (Shear) Dug.) in the alkaline soils of the Southwest has already continued for half a century. The literature involved has recently been reviewed by Rea (*16*)[3] and by Streets (*17*). Nearly all of these discussions have been from the viewpoint of plant pathology or agronomy. Garrett (*7*) included this disease in his broad discussions of root-disease problems. Observations pointing to a soil microbiological factor began with Pammel's (*14*) observation that the incidence of this disease was lessened by liberal use of stable manure. Actual predication of soil microorganisms as a factor in the conquest of the disease was offered by King and his colleagues (*8, 9*) in a series of papers upon the manure treatment of root rot plots in Arizona, beginning in 1923 and definitely proposing the microbiological interpretation of such control in 1934. Search for antagonistic organisms effective in the cotton field has thus far proved futile (Morrow et al., *12* and unpublished data), although such organisms are readily demonstrated in pure culture. Up to the present, no antibiotic agent has been able to destroy or render static a root rot fungus already established as a parasite in the cotton plant. The work discussed in this paper, therefore, deals with the relations of the soil micropopulation to the root rot fungus in the soil and in dead or decaying roots, not in its active phase as a parasite.

The persistence of *Phymatotrichum omnivorum* on cotton roots under different soil or root conditions and the interpretation of the observed differences have remained subjects for speculation. King and Loomis (*10*), McNamara and Hooton (*11*), and Ratliffe (*15*) have observed that the parasite is viable for considerable periods after its invasion of cotton roots. That its viability is decreased under certain conditions is also known. Rea (*16*) has stated that the older fungus strands on diseased and dying portions of cotton roots gradually exhaust the available food materials therein and die with the roots to which they are attached. Ezekiel (*5*) has noted decreased survival percentages of *P. omnivorum* on cotton roots following root cutting or girdling, as well as a decrease in alcohol-soluble solids contained in the roots. Such studies suggest that food exhaustion or changes in root composition become limiting factors for survival of the parasite.

The role of microbial interrelationships in determining the survival of plant pathogens has been reviewed by Garrard and Lochhead (*6*). King, Hope, and Eaton (*9*) have discussed the importance of microbial factors in the manurial control of cotton root rot on irrigated land. Clark and Thom (*4*) concluded that the suggested microbiological

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[3] Italic numbers in parentheses refer to Literature Cited, p. 547

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sanitation effects of manurial amendments must be accomplished through the agency of the soil microflora; the root microfloras themselves showed no appreciable changes following organic amendments. Recently Adams, Wilson, Hessler, and Ergle (*1*) reported that early fall plowing of cotton stalks checked the destructiveness of the fungus in the following cotton crop. The extent to which root microfloras influence the survival of *Phymatotrichum omnivorum*, and in turn the extent to which microfloras associated with cotton-root surfaces are influenced by plant injuries inflicted by early plowing or girdling, remain unreported.

It is known that sclerotia of the cotton root rot fungus may remain in field soil as viable and infectious material for many years, even when fields are maintained in nonsusceptible crops or in clean fallow. Sclerotia have been considered important in the survival of primary infection centers; their elimination, therefore, becomes desirable in soil sanitation.

Certain physical conditions, such as soil desiccation or aeration, have been reported to influence the survival of sclerotia (*8*, *13*, *18*). The successful manurial control of several soil-borne diseases (*7*) and the possibility that microbial relationships contribute to the elimination from soil of plant pathogens raise the question of the ability of sclerotia of *Phymatotrichum omnivorum* to survive when exposed to the microbial activity associated with fresh organic residues in soil.

It has been the purpose of the following investigations to consider (1) the growth and persistence of *Phymatotrichum omnivorum* on cotton roots or root segments under different environmental conditions and (2) the elimination of *P. omnivorum* sclerotia from soil, with especial reference to the periods of microbial activity following organic fertilizations.

EXPERIMENTAL PROCEDURE AND OBSERVATIONS

GROWTH AND PERSISTENCE OF PHYMATOTRICHUM MYCELIUM

On Cotton-Root Segments in Amended Laboratory Soils

Indications of the importance of the effects of environmental factors on *Phymatotrichum omnivorum* were readily seen when freshly infected roots were collected from field cotton and placed in contact with differently treated soil in the laboratory. A representative experiment is summarized briefly.

Ninety-six quart jars, in 4 batteries of 24 jars each, were half-filled with Hunt clay. The first battery was retained unamended; the second, third, and fourth batteries received 1 percent superphosphate, 3 percent barnyard manure, and 3 percent chopped sorghum fodder, respectively. Four jars in each battery were brought to optimum moisture content immediately and incubated at a controlled room temperature of 25° C. for 30 days. Additional sets of 4 jars in each battery were moistened at intervals, so that, after 30 days had elapsed, jars were available to represent periods of incubation of 0, 5, 10, 15, 20, and 30 days for each of the 4 soil conditions. All soil containers were then inoculated with *Phymatotrichum omnivorum;* 2 jars in each block of 4 received agar-culture inoculum, and the other 2 replicates in the block received infected root segments. Observations on the growth of *P. omnivorum* mycelium and on its persistence after development were made at 3, 5, 10, and 16 days after inoculation.

Mycelium of *Phymatotrichum omnivorum* developed quickly over the soil surfaces in jars containing unamended and superphosphate-amended Hunt clay. The mycelium produced showed little disintegration during an observation period of 16 days, and random subcultures to new check soils consistently showed viability of the fungus. In contrast, infected roots or agar inocula placed on soil containing organic materials either failed to grow or showed initial growth followed by disintegration of the mycelium. The soil lots amended with manure and with sorghum fodder differed somewhat both in their periods of mycelial inhibition and in their associated soil micropopulations. The presence of manure in the soil inhibited growth or persistence of *P. omnivorum* mycelium from inocula only within the first 15 days after its application; after incubation for 20 days or longer, manured soil permitted growth of mycelium. Sorghum fodder in soil prevented growth of the fungus for the full 30-day period following its application. For both amendments, the period unfavorable to mycelial growth showed close agreement with the period of intensified microbial activity. Greater and more prolonged differences were noted for the soil micropopulations following fertilization with sorghum residues; after 30 days the soil micropopulations in such soils were still approximately seven times those of check soil. For manured soil, micropopulations were slightly more than double those of the check soil for the first 2 weeks after treatment but showed little difference thereafter.

Other experiments, established under conditions similar to the preceding series but with different types of amendments, showed that decomposing organic materials generally, if capable of supporting increased microbial activity, induced situations in which mycelial growth from freshly infected cotton roots was inhibited completely or else destroyed rapidly after an initial development. Among other organic materials, cottonseed meal and hulls and chopped cotton roots were found to be suitable treatments for inhibiting mycelium.

ON COTTON ROOTS IN FIELD EXPERIMENTS

The response of the root-surface microflora to various types of root injury was studied in field experiments. In order to establish a background of information concerning normal root-surface micropopulations, before attempting to define those of injured roots, a seasonal study of the microfloras associated with cotton of different ages and of different dates of planting was started May 3, 1940, and was continued for 6 months. Field samples consisted of composites from a minimum of five plants; the root systems collected by excavations were separated into crown, deeper tap, and lateral portions for laboratory studies. Microbiological analyses were made according to the methods previously reported by Clark (*2*, *3*), except that whole root segments rather than root-surface scrapings were employed in the primary dilutions. Surface areas were determined as accurately as possible by measurements, and the microbial populations were expressed as number of micro-organisms per square centimeter of root surface. The seasonal microfloras associated with surfaces of uninjured cotton roots are shown in table 1.

TABLE 1.—*Microfloras associated with the surfaces of healthy cotton roots, season of 1940*

Date of planting cotton	Date of root sampling	Age of cotton sampled	Microbial groups determined														
			Total microbial number on—			Dye tolerant bacteria on—			Filamentous fungi on—			Spores of bacillus on—			Fluorescent bacteria on—		
			Crown roots	Tap-roots	Lateral roots	Crown roots	Tap roots	Lateral roots	Crown roots	Tap roots	Lateral roots	Crown roots	Tap roots	Lateral roots	Crown roots	Tap-roots	Lateral roots
		Days	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Hundreds*	*Hundreds*	*Hundreds*	*Hundreds*	*Hundreds*	*Hundreds*	*Hundreds*	*Hundreds*	*Hundreds*
Apr. 16	May 3	17	[1] 2,850			2,500			4.8			101.0			7,960.0		
	May 15	29	1,650			450			51.0			8.3			510.0		
	June 22	67	335	94	58	39	20		13.4	2.2	4.0	3.1	4.7	5.2	2.9	6.3	9.2
	July 5	80	522	127	22	194	16	4	2.7	.9	1.3	8.9	10.2	8.3	26.6	4.9	.1
	July 11	86	464	117	39	740	56	47	15.3	.8	.9	1.7	4.2	5.2	48.1	.1	1.3
	July 26	101	131	15	10	175	10	10	4.5	2.3	.3	3.3	1.9	3.7	16.1	.1	.1
May 7	July 11	65	608	150	138	382	263	36	1.9	2.0	1.7	2.7	4.7	2.0	48.4	.7	2.3
	July 18	72	414	91	101	197	42	71	1.1	.6	.4	9.6	4.3	5.2	74.4	.8	.9
	July 25	79	410	191	136	164	61	18	2.0	.7	.6	3.4	5.7	3.1	42.8	2.5	.2
	Aug. 15	100	34	124	11	63	147	14	3.2	.1	.3	4.7	4.0	2.4	17.6	1.6	1.2
June 4	June 22	18	333			37			13.0			8.5					
	July 5	31	1,259			355			2.7			5.9			30.9		
	July 11	37	1,280	283	160	1,375	358	100	330.0	2.4	2.7	4.4	6.0	1.5	920.0	20.8	37.6
	July 18	44	438	57	106	239	28	52	1.6	1.8	1.3	4.6	2.1	3.6	20.4	1.6	9.0
	July 25	51	577	54	243	193	11	35	2.0	.3	2.3	6.9	4.7	7.8	94.8	.5	3.0
	Aug. 15	72	60	17	19	48	14	30	1.9	.2	.4	1.6	1.1	1.3	9.0	.1	.2
	Sept. 20	108	278	91	275	181	35	222	3.1	1.4	2.8	8.7	4.4	7.3	1.7	.1	42.3
	Sept. 27	115	265	64	143	220	50	103	3.2	.8	.5	10.9	4.1	1.6	2.7	.4	1.7
	Oct. 18	136	293	109	128	230	72	92	3.5	1.7	2.0	7.4	5.3	2.3	41.5	1.9	2.2

[1] Number of micro organisms per square centimeter of root surface.

Young cotton plants possessed somewhat higher root micro-populations than did older plants; the latter, when maintained uninjured under the usual practices of continuous cotton cultivation, had root microfloras that did not differ materially during the late fall from those observed during the early fall or late summer. In contrast, greatly intensified microbial activity was observed for root surfaces of injured cotton plants. Representative data for different types of injury are presented in table 2. Injuries inflicted during the late fall had less effect on root-surface microfloras than did late-summer injuries. Relative increases of total microbial numbers following injuries to roots on August 19 over those for uninjured root systems are shown in table 3. Differences in the gross appearance of such roots were easily apparent (fig. 1).

TABLE 2. *Microfloras associated with the taproots of uninjured and injured cotton plants, season of 1940*

Date of sampling	Microbial groups determined	Micro-organisms per square centimeter of root surface of plants							
		Not injured	Cut 4 inches below crown			Cut 4 inches above crown			Girdled 4 inches above crown, Aug 19
			Aug 19	Sept 19	Oct 19	Aug 19	Sept 19	Oct 19	
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Aug. 19	Total microbial number	16,550							
	Dye-tolerant bacteria	14,400							
	Filamentous fungi	22							
	Spores of bacillus	105							
	Fluorescent bacteria	14							
Sept. 20	Total microbial number	90,500	14,500,000			350,000			8,800,000
	Dye-tolerant bacteria	35,000	14,200,000			112,000			6,000,000
	Filamentous fungi	140	825			230			68
	Spores of bacillus	141	1,049			737			548
	Fluorescent bacteria	3	72,900			25			66
Oct. 18	Total microbial number	109,000	17,200,000	80,000		55,000	72,000		231,000
	Dye-tolerant bacteria	72,000	16,400,000	26,000		55,000	47,000		156,000
	Filamentous fungi	174	1,098	1,679		65	97		1,390
	Spores of bacillus	526	448	644		227	767		1,307
	Fluorescent bacteria	193	462,400	65		28	154		1,173
Nov. 19	Total microbial number	28,000	7,100,000	319,000	53,000	95,000	92,300	32,000	885,000
	Dye-tolerant bacteria	39,300	4,700,000	345,000	53,000	7,300	80,300	43,700	158,000
	Filamentous fungi	560	4,700	600	100	90	46	60	330
	Spores of bacillus	870	4,800	2,100	1,100	520	850	440	2,200
	Fluorescent bacteria	1,800	128,400	81,300	78	200	29,800	400	300

TABLE 3. *Comparative total microbial counts on surfaces of uninjured and injured cotton roots at 1, 2, and 3 months after injury on Aug. 19, 1940*[1]

Date of sampling	Check (uninjured)	Cut below the crown	Girdled above the crown
Sept. 20	1	160	97
Oct. 18	1	158	2 2
Nov. 19	1	254	14

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[1] Micropopulations on surfaces of injured roots compared with those on healthy uninjured roots (taken as unity) on corresponding dates of sampling

ON COTTON ROOTS IN DIFFERENT STAGES OF DECOMPOSITION

In a concluding experiment, the availability of cotton roots at different stages of decomposition as nutrient material for *Phymatotrichum omnivorum* was determined. Cotton roots or root segments that had undergone periods of decomposition from 0 to 350 days were collected from 29 different laboratory and field experiments. Each collection of root material was divided into 3 equal parts; these were moistened and placed in Erlenmeyer flasks. The first flask of each set of 3 was left unsterilized; the 2 remaining flasks were autoclaved until sterile. One of these was maintained sterile thereafter, except for the addition of *P. omnivorum;* the other was inoculated with unsterilized soil. Standard agar disks of *P. omnivorum* were introduced into all flasks, and observations for growth of mycelium were made during the following 2 months. The source and treatment of the cotton roots employed are shown in table 4.

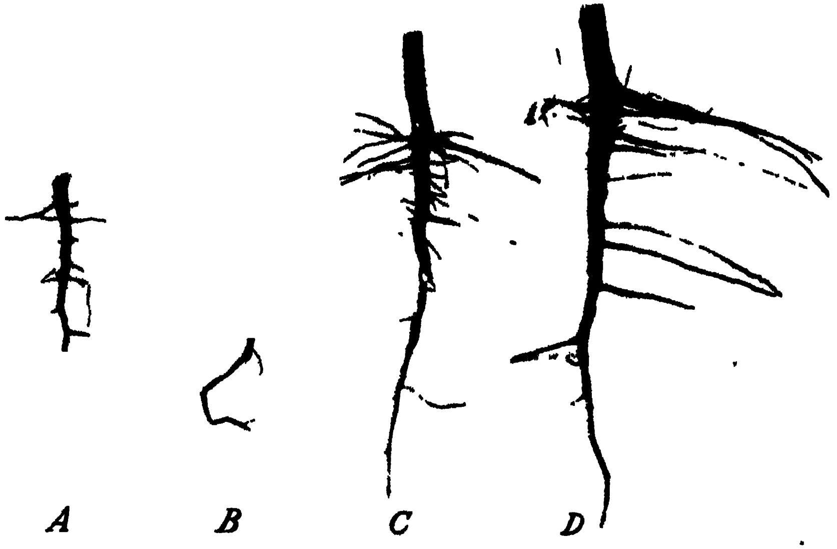

FIGURE 1.—Appearance of cotton roots injured on August 19, 1940, and excavated November 18, 1940: *A*, Clipped below the crown, *B*, girdled above the crown; *C*, clipped above the crown, *D*, uninjured cotton root (check) from the same experiment.

Without exception, in the unsterilized flasks *Phymatotrichum omnivorum* mycelium failed to develop upon any root material, regardless of the extent of decomposition previously undergone by the root segments. Mycelium developed in all flasks sterilized and then subsequently inoculated with *P. omnivorum* only; in such flasks the competitive effect of other micro-organisms was absent. Mycelium failed to develop in those flasks sterilized and then reinoculated with a pinch of nonsterile soil together with *P. omnivorum;* such results showed that the growth obtained in the pure-culture series did not occur simply because of the liberation of additional food materials during the sterilization process.

TABLE 4. *Types of cotton roots studied for their ability to support growth of Phymatotrichum omnivorum*

Condition of root material and treatment number	Treatment undergone by the cotton roots	Period of decomposition before experiment was established
Healthy:		*Days*
1	None	0
2	Buried in unamended soil	7
3	do	14
4	do	30
5	do	45
6	do	60
7	do	75
8	do	90
9	do	350
10	Buried in manured soil	350
11	Buried in sorghum fodder amended soil	350
12	Buried in 15-0-0 amended soil	350
13	Buried in 0-15-0 amended soil	350
14	Injured by cutting above crown and left in field soil	11
15	do	40
16	do	70
17	Injured by cutting below crown and left in field soil	11
18	do	40
19	do	70
20	Injured by girdling above crown and left in soil	70
Diseased:		
21	Buried in unamended soil	350
22	Buried in manured soil	350
23	Buried in sorghum fodder amended soil	350
24	Buried in 15-0-0 amended soil	350
25	Buried in 0-15-0 amended soil	350
26	Buried in unamended soil	273
27	Buried in manured soil	273
28	Buried in sorghum fodder amended soil	273
29	Buried in 0-15-0 amended soil	273

ELIMINATION OF PHYMATOTRICHUM SCLEROTIA FROM SOIL

LABORATORY EXPERIMENTS

The ability of *Phymatotrichum omnivorum* sclerotia to survive under different soil conditions in the laboratory was observed following the addition of known numbers of sclerotia to Houston and Hunt clays receiving different amendments. At intervals ranging from 10 days to 6 months, soil lots were examined for number of recoverable sclerotia and for microbial activity in the soil or on the sclerotial surfaces. Such studies showed that many more sclerotia were eliminated from soil containing fresh organic materials than from soil containing no such amendments.

When the data from five separate experiments, conducted during the course of 2 years, are considered collectively, the greater susceptibility of sclerotia in organic-amended soils is convincingly shown. In soil amended with barnyard manure or with chopped sorghum fodder, 64.9 and 69.7 percent of the sclerotia added were eliminated; in superphosphate-amended and in unamended soil, 14.4 and 19.6 percent, respectively, were eliminated. Other organic materials, such as cottonseed meal or hulls or chopped cotton roots, were found as effective as manure or sorghum fodder in reducing the sclerotial population in soil.

The results of a typical experiment are presented in table 5. The sclerotia used in this experiment were light brown-buff types washed out by hand from root rot-infested field soil; 95 percent of these sclerotia were found to be viable following field recovery. In other experiments analogous to the one recorded in table 5, the older dark-brown types of sclerotia recovered from field soil were employed; the results obtained with such sclerotia were essentially the same insofar as contrast between check and organic-amended soils was concerned, although in several experiments there appeared a tendency for the old dark-brown types to be more resistant generally. Preliminary washings revealed that the Hunt clay employed was originally free of sclerotia; and also that the screening and washing methods employed permitted complete recovery immediately following the addition of sclerotia from either amended or unamended soil.

TABLE 5.—*Recovery of Phymatotrichum omnivorum sclerotia*[1] *from soil in relation to periods of incubation and types of soil fertilization*

Incubation period (days)	Sclerotia recovered from				
	Unamended check	Soil fertilized with			
		1 percent superphosphate	3 percent manure	3 percent sorghum fodder	3 percent chopped cotton roots
15	190	186	76	77	51
35	162	188	58	53	48
45	153	175	58	42	67
60	165	169	45	63	39
75	152	161	55	45	46
Total	822	879	292	280	251

[1] Number recovered after incubation periods indicated. 200 sclerotia were originally added to each container.

It was observed repeatedly that major reductions in sclerotial number were secured only during the periods of intensive microbial activity in soil. Since the microbiological data collected for the different laboratory experiments are extensive, such material is presented in summary form. Soil (in unamended or inorganic-amended lots) in which sclerotia persisted with relative impunity showed total micropopulations ranging from 15 to 50 million, expressed as per gram of air-dry soil, throughout the periods of incubation employed. Soil receiving barnyard manure or chopped sorghum fodder at 3-percent rates showed micropopulations of from 100 to 300 million during the first 2 weeks after treatment; following their initial precipitous increases, such micropopulations declined more gradually, until they were comparable to those in untreated soil. Inspection of table 5 shows that major reductions in sclerotial numbers were secured during the initial period of intense microbiological activity. Figure 2 shows the decomposed and hollow-shelled sclerotia after 15 days' burial in fertilized soil lots, and, in contrast, the undecomposed, solid-appearing sclerotia recoverable after burial in untreated soil.

Direct microscopical studies of stained Cholodny slides (*9*) showed general confirmation of the cultural data, and in addition, yielded some further information concerning microbial activity on or near the surfaces of buried sclerotia. After short burial periods in soil, numerous fungus strands were seen in the sclerotium-glass slide contact region; certain fungus strands appeared as *Phymatotrichum omnivorum* mycelia. After longer incubation periods, shadowlike strands of fungus mycelia covered with masses of bacteria were observed, suggesting early colonization of the sclerotial surface region by fungus strands, followed in turn by bacterial invasion and decomposition.

Spores of *Alternaria*, *Hormodendrum*, and *Trichoderma*, and long slender conidia of *Actinomyces* were observed; this suggested that mycelial stages of these forms accounted for some of the fungus strands observed shortly after burial of sclerotia in soil.

Germination of *Phymatotrichum omnivorum* sclerotia was suggested by the Cholodny slide studies. The possible role of germination of sclerotia in the differences in survivals in organic-amended and in unamended soil (table 5) was investigated in the following experiments.

Eight replicates of 5 soil conditions (untreated, 1 percent superphosphate, 3 percent chopped green alfalfa, straw, manure) were established in laboratory containers, and 10 sclerotia were buried in each of the 40 containers. Two lots of sclerotia were employed; one lot consisted of younger, light-brown sclerotia, of 80-percent viability; the second lot, of old, dark-brown sclerotia, of 50-percent viability. Aliquots of each lot were killed by heating to 85° C. for 5 minutes. In the preparation of the experiment, duplicate containers in each of 8 replicates received (1) untreated young sclerotia, (2) untreated old sclerotia, (3) killed young sclerotia, and (4) killed old sclerotia.

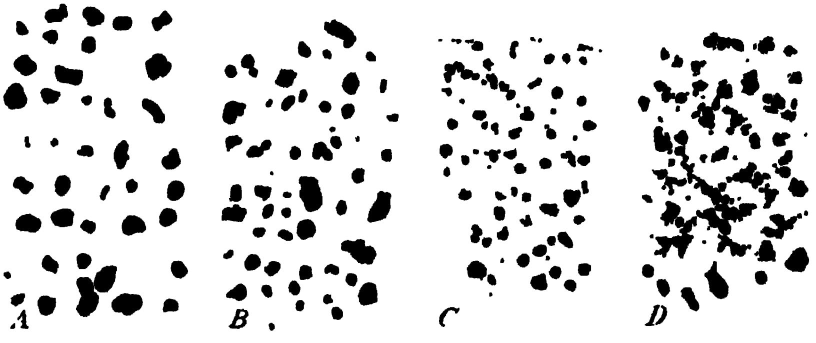

FIGURE 2.—Appearance of *Phymatotrichum omnivorum* sclerotia *A*, Prior to burial in experimental soil lots. *B–D*, Following burial for 15 days in (*B*) unamended Hunt clay, (*C*) Hunt clay plus 3 percent chopped sorghum, and (*D*) Hunt clay plus 3 percent chopped alfalfa. About ×1.

All containers were incubated for 17 days and then examined for condition of the buried sclerotia. As all sclerotia were placed at known locations on glass slides, full recovery was possible. Observations were recorded on the basis of whether recovered sclerotia were solid and intact, or were disintegrating or hollow shells. A summary of observations is presented in table 6.

TABLE 6. *Percentage of viable and killed sclerotia recovered from soil receiving different treatments*

Soil treatment	Young sclerotia		Old sclerotia	
	Living	Killed	Living	Killed
	Percent	*Percent*	*Percent*	*Percent*
Untreated (check)	20	5	15	0
Superphosphate	5	0	10	0
Ground straw	70	15	30	15
Ground alfalfa	75	30	40	25
Manure	75	40	15	5

FIELD OBSERVATIONS

A field experiment provided four areas on root rot-infested land. Two areas received either barnyard manure or sorghum fodder thoroughly mixed into the upper 8 to 10 inches of soil by rotary plowing on October 3, 1939; a third area received similar deep tillage without organic amendment on the same date; and the fourth area received the usual bedding later in the fall season. All areas were cropped to cotton during the 1940 season.

Observations for the occurrence of sclerotia in these areas were made by field excavations during the latter part of August 1940. No sclerotia were found in the upper 12 inches of soil in areas receiving deep tillage or organic materials; sclerotia were present below the 12-inch level. In the normally bedded area, sclerotia were observed in abundance in the 6- to 12-inch level. The largest proportion of dead cotton plants occurred in this check area, and the smallest proportion in the manured area (table 7). Increased micropopulations were apparent in all the field areas after the first good fall rain in October 1939, but periods of prolonged microbial activity were noted only in areas receiving organic manures. Total micropopulations in the soil at five different dates of sampling, as well as dead cotton in the four areas at three different dates of mapping, are shown in table 7.

TABLE 7.—*Soil micropopulations and incidence of dead cotton in differently treated field areas*

TOTAL MICROBIAL NUMBER[1]

Dates of sampling or mapping	Plot treatment			
	Normal bedding	Deep rotary tillage	Deep tillage + barnyard manure	Deep tillage + sorghum fodder
	Millions	*Millions*	*Millions*	*Millions*
Oct 13, 1939	159.5	335.5	452.6	287.9
Nov. 29, 1939	10.2	26.1	62.3	115.1
May 4, 1940	18.6	19.8	118.3	222.1
June 22, 1940	12.2	12.5	50.6	50.0
July 20, 1940	17.1	21.2	36.8	47.9

INCIDENCE OF DEAD COTTON PER PLOT[2]

Dates of sampling or mapping	Normal bedding	Deep rotary tillage	Deep tillage + barnyard manure	Deep tillage + sorghum fodder
	Percent	*Percent*	*Percent*	*Percent*
September 1939	85.3	80.0	96.0	74.0
August 1940	90.0	36.3	7.0	20.0
October 1940	94.0	69.0	34.0	60.0

[1] Expressed as millions per gram of air-dry soil

[2] Expressed as percentage of total stand

DISCUSSION

It is known that under field conditions *Phymatotrichum omnivorum* makes an extensive mycelial growth during the late summer and early fall; excavation studies during the fall season may reveal the parasite on the roots of many cotton plants even though no aboveground symptoms of the disease are evident. If the mycelial stage is recognized as capable of development upon a host-plant root system, and, in contrast to the saprophytic flora, of attacking healthy roots, but if at the same time it is known to be incapable of successful competition with the large micropopulations engaged in the decomposition of fresh organic residues, the importance of ending the sheltered host-borne stage of the parasite as quickly as possible and thus prolonging the period during which it is subject to attack or competition of other soil micro-organisms becomes evident.

Cotton plants injured by late-summer clipping or girdling showed greatly intensified root-surface microfloras. Since the mycelium of *Phymatotrichum omnivorum* was inhibited or destroyed in organic-amended soils in which the increases in micropopulations over those of check soils not destructive to mycelium were of the order of 5 to 10 times, it is not improbable that the microbial activity on root surfaces, where the micropopulations increased as much as 100 to 200 times, provided an environmental factor unfavorable to the fungus. Growth of *P. omnivorum* from inocula on injured or decomposing cotton roots at practically any stage of decomposition, following sterilization to remove competitive microbial activity, further supports this reasoning. The limitation of survival of *P. omnivorum* mycelium on decomposing cotton roots is considered, therefore, more directly a matter of microbial interrelationships than of food exhaustion.

Phymatotrichum sclerotia, in contrast to the mycelium, appear very resistant to a prolonged soil-borne stage of existence; their longevity under fallow and different crop-rotation schemes is generally recognized. That sclerotia are susceptible to elimination from soil during periods of intensive microbial activity following applications of fresh organic residues is shown by the present work. The contrast between persistence of sclerotia in organic-amended and unamended soils within the first 10 to 15 days after burial was at first difficult to understand, and it was only after the Cholodny slide studies and the burial of heat-killed sclerotia that the increased germination of sclerotia under soil conditions favoring general microbial activity offered a partial explanation of the difference in survival. In the absence of a host plant, the emerging mycelium becomes exposed to saprophytic conditions, and the parallel results noted during the course of mycelium and sclerotia studies become understandable. It is not known why heat-killed sclerotia are more rapidly eliminated from organic-amended soil during the early period of incubation. It is possible that a mass-inoculation effect with more numerous and perhaps more diverse soil micro-organisms is obtained; it is also possible that the sclerotia substance presents a source of supply of one or more elements required during the initial decomposition stages of the added organic material by the soil microflora concerned.

The field observations on the absence of sclerotia in the organic-amended and tilled areas, together with the decreased incidence of dead cotton as shown by the August 1940 mapping, indicated that reduction in infectious material could be obtained in the field. However, it was not apparent from the work performed whether the more significant factor was (1) the elimination of root-borne mycelial strands important either directly as subsequent sources of infection or indirectly through their later production of sclerotia or (2) the elimination of soil-borne sclerotia formed prior to fertilization and tillage. If the former factor is the more significant, the destruction of roots by early plowing becomes more important than the application of additional organic residues to soil. Clark and Thom (4) have expressed the opinion that any microbiological sanitation effects of added organic residues are accomplished through the agency of the soil microflora, since such fertilizations failed to affect the root microfloras of healthy cotton. If the destruction of soil-borne sclerotia is essential, the value of adequate mixing of fresh organic materials into soil to secure the greatest possible contact with soil-borne infectious material is apparent.

SUMMARY

Hunt clay to which no organic material was added permitted growth and persistence of *Phymatotrichum omnivorum* mycelium over the soil and root surfaces in open containers inoculated with segments of recently infected cotton roots, when maintained under favorable moisture and temperature conditions. Soil containing added organic matter, but otherwise similarly inoculated and maintained, either completely inhibited the growth of *P. omnivorum* or permitted initial growth which was followed by disintegration of mycelium.

Under field conditions, cotton roots injured during the late summer or early fall showed pronounced increases in micropopulations associated with root surfaces; such increases were proportionately greater than those caused in soil by organic amendments and found inimical to the growth of the parasite.

The majority of *Phymatotrichum omnivorum* sclerotia buried in organic-amended soil were quickly destroyed; in contrast, the great majority of sclerotia buried in soil without organic residues persisted. Sclerotia remaining after subsidence of the microbial activity occasioned by fertilization persisted with little further reduction in numbers for several months; for the elimination of either mycelium or sclerotia, treatments with organic materials were especially effective during the period of increased microbial activity.

Removal of the competitive effects of other microbes by soil sterilization, and subsequent reinoculation with *Phymatotrichum omnivorum*, permitted good growth of fungus mycelium regardless of the amount of decomposition which the cotton roots or organic residues employed had previously undergone. Fungus survival was considered, therefore, to be limited by microbial interrelationships rather than by food exhaustion.

It was observed that during the early stages of incubation viable sclerotia disappeared more rapidly from amended soils than killed sclerotia; the importance of sclerotia germination as one factor in soil sanitation was thus suggested. That other factors were operative also was suggested by the greater elimination of heat-killed sclerotia from organic-amended soil than from unamended check soil

Following field application of organic materials, together with early October plowing, increased microbial activity, reduction of the incidence of dead cotton in the succeeding crop, and greater difficulty of sclerotia recovery from the amended levels in field soil were observed.

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DISSEMINATION OF FUNGI THAT STAIN LOGS AND LUMBER[1]

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INTRODUCTION

In the literature on wood-staining fungi there are several descriptions (*2, 4, 5, 6, 7, 12, 13*)[3] of close associations of certain species of staining fungi with certain bark beetles and their tunnels, mostly in relation to stain in standing timber. There is, however, practically no general information on the means of dissemination of staining fungi to logs and lumber or the relative importance of these means. In fact, except for the work on bark beetle dissemination, most statements referring to dissemination of staining fungi seem to be based largely on supposition.

During the past 10 years considerable data on the dissemination of fungi that stain logs and lumber have been accumulated by the Division of Forest Pathology at its New Orleans branch. Brief accounts of some of these findings have been published (*9, 11*). Although the present studies are far from exhaustive they probably give an adequate picture of dissemination insofar as it bears on practical control of stain in logs and lumber in Louisiana and Mississippi. Although the study was limited to these two States, there is no reason to believe that the data would not be applicable to other States in the Southern Pine Belt.

MEANS OF DISSEMINATION

AIR CURRENTS

In an attempt to determine the prevalence of air-borne spores of staining fungi in mill yards, malt-agar plates were exposed for $\frac{1}{4}$, $\frac{1}{2}$, 1, and 2 minutes, most often for 1 minute. In table 1 are listed the fungi secured in this manner. For convenience of comparison the results were transformed to the uniform basis of 125 plates exposed for 1 minute.

It is evident from table 1 that the number of air-borne spores of the important staining fungi in mill yards at a given time is not great but that the number of spores of some of the less important staining fungi is high. These results are based on exposures made in yards of average to good sanitation; none of the yards had severe staining of lumber at the time exposures were made. Because of the large

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[3] Italic numbers in parentheses refer to Literature Cited, p. 557.

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TABLE 1 — *Fungi developing in malt-agar plates exposed in lumber-seasoning yards and inside mills*

Fungus	Fungi (calculated) per 125 plates exposed for 1 minute in—		
	Hardwood yards [1]	Pine yards [2]	Mills
	Number	*Number*	*Number*
Important staining fungi			
Ceratostomella ips Rumbold	0	0	4
Ceratostomella pluriannulata Hedg	1	0	162
Endoconidiophora coerulescens Munch	2	0	15
Graphium rigidum (Pers ex Fr) Sacc	0	0	55
Helminthosporium spp	3	1	0
Minor staining fungi			
Alternaria spp	19	7	7
Cladosporium spp	116	70	187
Pullularia spp	103	132	1
Torula spp	3	0	0
Dark, undetermined [4]	21	5	0
Nonstaining organisms	688+	380+	855+

[1] Based on 145 plates exposed at 4 mills on 11 different dates during 1937 and 1938

[2] Based on 105 plates exposed at 4 mills on 7 different dates during 1937 and 1938

[3] Based on 17 plates exposed in 2 mills on 4 different dates in December 1937 Mills were cutting mostly hardwoods at the times exposures were made

[4] Some of the staining fungi listed may be included, but if so they were obscured by molds and bacteria

number of nonstaining fungi present, the agar-plate exposure method entailed excessive culture work in separating the developing colonies Therefore this method was dropped in favor of exposures of sterile wood blocks

Pine blocks approximately 1×1×5 inches were immersed for ½ minute in boiling water, and placed in sterile jars until exposed At each of 2 mills, on 4 occasions, 20 of these blocks were suspended by wires attached to nails in the ends of the blocks These mills were in central Mississippi and were approximately 50 miles apart One mill had a clean yard with practically no stain, the other had a yard with a large pile of slabs at the edge that were green and there was heavy stain in much of the lumber The blocks were exposed for 20 minutes and watched continuously for insect visitors All blocks touched by insects were discarded After exposure the blocks were

TABLE 2 *Fungi developing on pinewood blocks exposed for 20 minutes in mill yards with light and heavy stain infection*

Fungi isolated	Blocks yielding fungi listed at indicated mill yard and date of exposure									
	Yard with light stain					Yard with heavy stain				
	May 11	June 23	July 28	Aug 30	Total	May 11	June 23	July 28	Aug 30	Total
Important staining fungi	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Ceratostomella pilifera (Fr) Wint	0	0	0	0	0	0	13	0	0	3
Ceratostomella ips Rumbold	0	0	0	0	0	0	0	14	0	3
Helminthosporium spp	18	0	36	7	14	0	6	21	22	10
Minor staining fungi										
Alternaria spp	55	18	43	43	37	42	0	14	22	21
Cladosporium spp	91	6	36	7	30	79	0	36	11	36
Pullularia spp	45	18	0	7	16	16	0	0	33	10
Dark undetermined [1]	0	12	29	21	16	11	13	14	44	17
Nonstaining organisms	91	100	100	79	93	95	100	100	100	98
	No	*No*	*No*	*No*	*No*	*No*	*No*	*No*	*No*	*No*
Blocks exposed	11	17	14	14	56	19	16	14	9	58

[1] Some of the staining fungi listed may be included, but if so they were obscured by molds and bacteria

returned to their jars. On 3 of the 4 exposure days humidity was high. For each test 6 blocks were left unexposed as checks. Although molds occasionally developed on these checks, no staining fungi were found.

The fungi cultured from these blocks are listed in table 2. As in the case of agar-plate exposures, it is evident that the populations of spores of important staining fungi in the air were not great during the exposure periods, even in the yard with heavy stain and under humidity conditions favoring spore production and discharge. On other occasions *Diplodia natalensis* Evans and *Graphium rigidum* have been secured from the air, showing that all the important staining fungi of the Southern States (*10*), with the possible exception of *Diplodia* sp., are at times air-borne.

By far the most important staining fungi in the Southern States are *Endoconidiophora coerulescens* and *Ceratostomella pluriannulata* on hardwoods and *C. pilifera* and *C. ips* on pine. From the data presented it might seem at first glance that air dissemination of these species is of very little importance. However, it must be remembered that green wood in air-seasoning piles is in a condition favorable for infection over a period of days or weeks, depending on weather conditions. If the data are reexamined in the light of this fact, i. e., if the time were days or weeks, instead of minutes as given in the tables, it is evident that appreciable numbers of spores can settle on lumber in piles during the period of susceptibility to infection by staining fungi. Since there are marked convection currents in new seasoning piles, air-borne spores can be carried throughout the piles.

INSECTS

At 7 mills in Louisiana and Mississippi, on 20 occasions from 1937 to 1940, insects found on or in seasoning lumber or stored logs were collected in gelatin capsules, and, as soon as practicable, were transferred to malt agar. The fungi isolated from insects in this manner are listed in table 3.

In evaluating the importance, as stain disseminators, of the various insects listed in table 3, three factors must be considered: (1) The importance of the fungus species carried, (2) the habits of the insects, and (3) the abundance of the insects on green wood. Because of the first of these factors a line was added to table 3 giving the percentage of the various insects cultured that yielded important staining fungi. It will be noted that in the groups labeled "Other beetles," "Dipterous insects," and "Other insects," an appreciable number carried important staining fungi. However, individuals cultured in these groups were of species that are not primarily associated with wood but are chance visitors to lumber seasoning piles and ordinarily do not penetrate seasoning piles. Therefore they do not inoculate wood in the parts of piles where staining may be severe, and probably are of little importance in disseminating staining fungi to seasoning lumber. These insects probably become contaminated with spores from lumber ends stained prior to sawing and from logs.

The important insect disseminators of staining fungi are probably to be found among ambrosia beetles, bark beetles, powder-post beetles, and certain other insects whose life habits bring them in close contact with wood.

TABLE 3.—*Fungi isolated from insects collected in or on lumber*[1] *in seasoning yards*

Fungi isolated	Insects yielding fungi													
	Ambrosia beetles				Bark beetles			Miscellaneous insects						
	Platypus compositus	*Pterocyclon mali*	*Xyleborus affinis*	Undetermined	*Ips* spp.	*Orthotomicus caelatus*	*Pissodes* sp.	*Xylobiops basilaris*	Tenebrionids	Staphylinids	Other beetles	Dipterous insects	Ants	Other insects
Important staining fungi:	*Pct*	*Pct*	*Pct*	*Pct*	*Pct*	*Pct*	*Pct*	*Pct*	*Pct*	*Pct*	*Pct*	*Pct*	*Pct*	*Pct*
Ceratostomella ips	0	0	0	0	62	75	0	0	0	0	0	0	0	0
Ceratostomella pilifera	0	0	0	0	0	25	0	0	0	0	0	0	0	13
Ceratostomella pluriannulata	3	13	2	35	0	0	0	5	50	0	11	10	9	7
Diplodia natalensis	0	0	0	0	0	0	0	0	25	0	0	0	0	0
Endoconidiophora coerulescens	2	3	9	0	0	0	0	5	25	0	0	6	0	0
Graphium rigidum	5	10	0	0	3	0	0	45	75	15	6	3		27
Helminthosporium spp.	2	3	2	0	0	0	0	0	0	0	28	16	0	0
Total	10	27	11	35	64	100	0	55	100	15	39	32	[illegible]	33
Minor staining fungi:														
Alternaria spp.	0	0	0	0	3	0	27	10	0	0	17	13	9	0
Ceratostomella multiannulata Hedgc. and Davidson	0	0	0	0	0	0	0	0	0	0	0	6	0	0
Ceratostomella spp.	0	3	0	0	0	0	0	20	0	0	0	6	0	0
Cladosporium spp.	11	0	0	10	5	50	60	40	0	14	61	52	36	[illegible]
Endoconidiophora moniliformis (Hedgc.) Davidson	5	7	2	20	0	0	0	20	2	91	22	0	0	[illegible]
Pullularia spp.	8	7	0	0	5	0	17	10	0	5	33	29	0	27
Dark undetermined	7	3	6	5	13	25	13	5	0	0	17	13	5	13
Nonstaining organisms	93	93	100	100	100	100	100	90	50	100	94	100	82	100
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Insects cultured	61	30	45	20	39	4	15	20	4	22	18	31	[illegible]	1

[1] Some of the ambrosia beetles were taken from galleries in newly infested logs.
[2] Some of the listed staining fungi may be included, but if so they were obscured by molds and bacteria.

AMBROSIA BEETLES

It has been shown (*9*) that in applying the common stain-control chemicals to stored hardwood logs, stain is not prevented if ambrosia beetle attack is heavy. Because of this it seems that ambrosia beetles must be common and effective carriers of staining fungi. Since ambrosia beetles heavily attack not only logs but also freshly sawed lumber of the most stain-susceptible hardwood species, such as sweetgum, they are also important in inoculating lumber. This inoculation is particularly effective, since the attacking adult bores deeply into the wood where the staining fungus would not be affected by any surface chemical treatment. The stain referred to in this discussion is not the restricted blackening around the tunnels of certain ambrosia beetles but the common stain found in sapwood of many tree species without beetle attack. Of the 176 adult ambrosia beetles cultured (*Platypus compositus* Say, *Xyleborus affinis* Eich., *Pterocyclon mali* Fitch, and undetermined ambrosia beetles), 16 percent yielded important staining fungi. Although this percentage is not high, it seems likely that ambrosia beetles are important stain disseminators since they attack both hardwood logs and lumber in large numbers.

BARK BEETLES

The close associations of certain wood-staining fungi with *Ips* (*2, 4, 5, 6*), *Dendroctonus* (*5, 6, 7*), and *Scolytus* (*12, 13*) have been demonstrated. Most of this work concerns associations of fungi and beetles in standing timber, although some of it was done with felled timber (*4*).

During the present investigations the only important bark beetles encountered were *Ips*[4] and its close relative, *Orthotomicus caelatus* (Eich.). A high percentage of the adults of both genera cultured yielded *Ceratostomella ips*, and the probability is that all individuals carry this fungus. On one occasion fresh *Ips* engravings in a pine log that was as yet unstained yielded *Diplodia natalensis* and on another occasion *Diplodia* sp. Furthermore, from adult *Ips* collected in mill yards, *Graphium*, *Alternaria*, *Pullularia*, and *Cladosporium* were isolated; and from adult *O. caelatus* from mill yards, *Ceratostomella pilifera*, *Pullularia*, and *Cladosporium* were isolated in addition to *Ceratostomella ips*, which is the specific staining fungus associated with *Ips* spp. Both *Ips* and *Orthotomicus* are attracted in large numbers to fresh pine lumber in seasoning piles during the flight season and were observed penetrating deeply into the piles. When wanes are present, bark beetles penetrate through the bark and can thus inoculate below the chemical dips used for stain control. However, inoculation may also occur merely by spores left when the adults walk on the lumber surface. This is the reasonable explanation of the occurrence of *C. ips* in test lumber, previously reported (*10*). Probably the most important inoculations accomplished by bark beetles are in freshly cut pine logs, which are frequently attacked in large numbers when stored for only short periods. Bark beetle infestations in logs probably have increased with the present practice, in the South, of using dry log storage instead of storage in millponds as was previously common. During the present investigations most of the serious pine log stain observed was associated with *Ips* and *Orthotomicus* infestations.

When periodic outbreaks of *Dendroctonus frontalis* Zimm. occur in standing timber (*8*), it seems likely that this beetle is an important disseminating agent of *Ceratostomella pini* in mill yards as well as in trees and logs. There is also the possibility that the air may be full of spores from dead trees during active outbreaks, such as the recent one in eastern Virginia, near mills of the Camp Manufacturing Co. The close association of this beetle and the staining fungus *C. pini* has been demonstrated (*5*).

The only other bark beetle noted during this study was a species of *Pissodes* that was sometimes found tunneling into wanes on pine lumber. No important staining fungi, however, were isolated from the 15 adults collected on several occasions. This beetle was not abundant. There are other genera of bark beetles that attack green logs and probably carry staining fungi to some extent.

POWDER-POST BEETLES

No study was made of the *Lyctus* group, as these insects mature in wood too dry for stain development. However, one of the powder-post beetles encountered, *Xylobiops basilaris* (Say), attacks green pecan, persimmon, and ash lumber. On one occasion 20 adults of *X. basilaris* were captured on or as they started to penetrate green pecan lumber, and 55 percent were found to be carrying important staining fungi. This high percentage was surprising, since at the mill where the beetles were captured they were observed only in pecan and ash woods, in which staining is not a problem, and since, so far as is known, *X. basilaris* requires at least 4 months to complete its life cycle. In 4 months most seasoning lumber would be too dry to support any luxuriant growth of staining fungi, and consequently emerging adults would not be heavily contaminated with such fungi. Little is known of the habits of this beetle, but it is possible that after emergence the adults from which the cultures were made had visited stained sweetgum or other lumber bearing a heavy growth of staining fungi, or that the adults had emerged from logs where the moisture content remained sufficiently high during the life cycle of the insect for abundant fruiting to occur in the tunnels at the time of emergence. Only if it can be shown that *X. basilaris* commonly visits the more stain-susceptible species of wood in its journey to the wood species in which it breeds can it be considered an important agent of dissemination. The number of this beetle observed was not large.

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[4] Species not determined

Miscellaneous Insects of Possible Importance

When lumber or logs support growths of staining or molding fungi on the surface, there are commonly present large numbers of adults of staphylinids, a small predaceous carabid, and tenebrionids, and also a small dipterous larva. These insects wallow in the fungus growth and undoubtedly become covered with the spores of the fungi fruiting on the wood surface. Apparently little is known of the habits of these insects and the extent to which they migrate to fresh unstained wood. The staphylinids and tenebrionids, however, have been observed in appreciable numbers in bulk-piled green lumber that was waiting to be stacked in seasoning piles. The values in table 3 for these insects are based on isolations from adults captured on bulked lumber.

MITES

Mites are common in lumber-seasoning piles. Although no isolations were attempted from them it is conceivable that they cause considerable secondary spread of staining fungi within a given pile. Mites occurring under bark, boards, slabs, etc., are known often to carry spores (*3*).

MILLING MACHINERY

Lumber may be heavily inoculated during the milling operation. A certain amount of fruiting of staining fungi commonly occurs on logs, particularly at the ends of hardwood logs. Inoculum from the ends of logs is thrown into the air by saws and also carried by saws and rollers to the surface of lumber coming in contact with them. In table 1 are listed the results of exposure of agar plates inside mills. The two mills in which exposures were made were cutting mostly hardwoods at the time the tests were made. The log supplies were average— not heavily stained. It is evident from table 1 that spore populations inside mills are large as compared with those in seasoning yards.

That untreated wood leaves mills inoculated with spores of staining fungi was further demonstrated by incubating in sterile jars pieces of bright wood collected at the edger and end trimmer. Of 25 pieces collected during the summer and winter of 1937, 20 stained heavily with *Endoconidiophora coerulescens*, *Ceratostomella pluriannulata*, and *Graphium rigidum* on hardwoods, and *C. ips* and *C. pilifera* on pine. When only logs apparently free of stain were being cut, about one-half the blocks of both pine and hardwood remained stain-free, but when stained logs were being cut all the blocks stained heavily even though the test material was taken several feet from visible stain or from stain-free logs.

RAIN WATER

Staining fungi have been caught in rain water dripping from lumber piles. In a properly constructed pile, water-carried spores are probably of little importance since rain water merely runs over the outside of the pile, inoculating wood that usually dries too rapidly for much stain development.

INFECTED WOOD

Dissemination by the transport of infected wood probably has a practical bearing only in the case of stained green cross stickers used in seasoning-pile construction. At some small mills, untreated green edgings are used for cross stickers and frequently are infected before they are used in the pile. "Sticker stain," resulting from the direct transfer of the staining fungus from the sticker to the lumber in contact with it, is common in these cases. Transfer from stained to unstained wood by direct contact has been experimentally secured with *Ceratostomella ips*, *C. pilifera*, *Diplodia natalensis*, and *Endoconidiophora coerulescens*. Other fungi were not tried.

RELATION OF CHEMICAL CONTROL TREATMENTS TO DISSEMINATION

It is now a common practice to dip freshly sawed lumber in a toxic chemical solution prior to air seasoning (*9*). Undoubtedly this practice has markedly changed the importance of some means of dissemination. Fungus spores disseminated during the milling operation are supposedly killed in passing through chemical dips. Likewise, air-borne spores and those carried by insects lighting and crawling on treated lumber are probably rendered largely ineffective by any toxic coating on the surface of the lumber. During prolonged periods of wet weather, when chemical treatments may fail to protect wood from staining, spores carried by air currents probably still play an important part in the dissemination of staining fungi. Since chemical treatment of exposed wood on log ends is the exception rather than the rule, dissemination to these ends by means of air currents and nonpenetrating insects is still relatively important.

Chemical dips have affected not only the importance of primary inoculations in fresh lumber but also secondary spread within seasoning piles by reducing the amount of inoculum present. At 28° to 30° C. the time between inoculation and production of new spores on untreated green wood was 46 hours for *Ceratostomella ips*, 22 hours for *C. pilifera*, 22 to 70 hours for *C. pluriannulata*, 22 to 46 hours for *Endoconidiophora coerulescens*, and 22 hours for *Graphium rigidum*.

These spores, resulting from primary infections, can be carried by various means to other parts of the same lumber pile before the surface of the wood is too dry for infection. Although similar values are not available for sporulation on treated wood, it can be assumed that most primary inoculations would never cause infections and that where infection occurs the period before new spore production would be longer than on untreated wood, resulting in much less secondary spread within the pile.

From observations made during the testing of commercial stain-control chemicals (*9*) it was obvious that these chemicals had little effect as insecticides against ambrosia beetles. Although based on less evidence, there are indications that stain-control chemicals likewise have little deterrent action on bark beetles boring into bark fragments left on treated lumber or on beetles crawling on treated lumber. Thus the chemical treatments probably have had little effect on spores carried by ambrosia and bark beetles where inoculation is made below the treated surface. Treatment, however, would reduce the importance of inoculations made by these beetles on the wood surface where they light and crawl prior to penetration.

DISCUSSION AND SUMMARY

Staining fungi are disseminated by means of air currents, insects, milling machinery, rain water, and the transport of infected wood.

Of the important staining fungi, *Ceratostomella pilifera*, *C. ips*, *C. pluriannulata*, *Endoconidiophora coerulescens*, *Graphium rigidum*, and *Diplodia natalensis* were found to be carried by air and insects; *C. pilifera*, *C. ips*, *C. pluriannulata*, *E. coerulescens*, and *G. rigidum* by milling machinery; and *C. pilifera*, *C. ips*, *E. coerulescens*, and *D. natalensis* by the transport of infected wood. Although most staining fungi are disseminated by various means, *C. ips* and probably *C. pini* are disseminated mostly by the bark beetles with which they are specifically associated.

Air-borne spores are now of less importance than before chemical treatments were in common use. However, they probably remain important since they may infect log ends, untreated lumber at the few mills not employing dips, and treated lumber during prolonged wet periods, when chemical treatments are not particularly effective.

Although many species of insects commonly carry staining fungi, the effectiveness of inoculations by most species is lessened by the fact that inoculations are in the outer parts of seasoning piles, where the lumber dries too rapidly for much stain development, and by the use of chemical treatments. However, two groups of insects, the ambrosia beetles and the bark beetles, remain of practical importance as carriers of staining fungi. Bark beetles are important mainly in inoculating pine logs, and ambrosia beetles in inoculating hardwood logs. Ambrosia beetles also attack green lumber of species most susceptible to staining, inoculating it below protective surface chemicals. All the indications are that none of the commercial stain-control chemicals in common use have any repellent effect on ambrosia and bark beetles. For the control of stain there is need of cheap and effective methods of repelling ambrosia and bark beetles from logs and the former also from lumber. A start has been made toward filling this need (*1*).

Dissemination and inoculation during the milling process are important when no chemical treatment is applied to the lumber as it leaves the mill. This importance increases when stained logs are being milled. Since chemical treatments are now commonly used to reduce stain infection, spores disseminated within mills are probably of little importance.

Spores carried by rain water probably are not of much importance, since in well-constructed seasoning piles water does not penetrate the interior of the piles, where most serious staining occurs.

Dissemination by the transport of infected wood may be important in causing "sticker stain," when stained green cross stickers are used in constructing seasoning piles.

In the practical application of data on dissemination, it is necessary to consider not only the agents of dissemination but also the sources of inoculum. It was pointed out that the general use of stain-control chemicals has probably greatly reduced the amount of inoculum of staining fungi in seasoning yards. Previously reported observations (*11*) indicate that slabs, edgings, and similar green material are suited to the production of spores of staining fungi in large numbers but that old and weathered debris is not. Therefore, sanitation in and around seasoning yards should include restriction of accumulations of green refuse of both pine and hardwood. With hardwoods, fruiting of staining fungi is also common on the ends of logs and the ends of lumber cut from stained logs (*10*). This argues for quicker utilization of hardwood logs and wider use of chemical treatments for hardwood log ends (*9*).

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EFFECT OF DIFFERENT SOIL COLLOIDS ON THE TOXICITY OF SODIUM SELENITE TO MILLET[1]

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INTRODUCTION

In a previous study it was shown that the toxicity of sodium selenate for millet and wheat is very little affected by different soil colloids (*7*).[2] It was pointed out, however, that the results obtained with sodium selenate should not be taken as applying to other forms of selenium, since preliminary experiments indicated that the selenite ion behaves quite differently from the selenate. The results reported in this paper deal with the effects of different soil colloids on the toxicity of sodium selenite.

METHODS

The toxicity of sodium selenite was measured chiefly by the growth of foxtail millet (*Setaria italica* (L.) Beauv.), although some experiments were conducted with Marquis wheat (*Triticum aestivum* L.) and with rice (*Oryza sativa* L.). The plants were grown in pure quartz sand and in mixtures of soil and sand containing usually 1 percent of colloidal soil material. Quantities of sodium selenite near the quantity required to reduce the yield one-half were applied to the two mediums, and the exact quantity of selenite required for half injury was determined from a graph of yields plotted against selenite applications. The value for quartz sand subtracted from the value for a sand-soil mixture gave a figure representing the effect of the soil application on selenite toxicity. As in previous publications (*6*, *7*), this effect of the soil was assumed to be due to the colloidal material present.

The fertilizer mixture added to each 1-gallon pot containing about 5 kg. of quartz sand or sand-soil mixture was 0.93 gm. of potassium nitrate, 0.33 gm. of ammonium sulfate, 0.0185 gm. of ferric tartrate, 0.42 gm. of magnesium chloride ($MgCl_2.6H_2O$), 0.085 gm. of sodium chloride, 0.0015 gm. of manganese sulfate ($MnSO_4.2H_2O$), and quantities of monocalcium phosphate that varied according to the estimated capacities of the soil for rendering phosphate unavailable (*5*). In most of the experiments 0.12 part per million of copper, 0.06 p. p. m. of zinc, and 0.1 p. p. m. of boron were also added. These salts, as well as the sodium selenite, were dissolved in the 750 cc. of water making up

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[1] Received for publication May 27, 1941.

[2] Italic numbers in parentheses refer to Literature Cited, p. 580.

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the water content of the pot and added to the top of the pot. The fertilizer added to the whole soils in experiments 1, 2, and 3 was 1.4 gm. of potassium nitrate, 0.33 gm. of ammonium sulfate, 0.136 gm of ammonium chloride, and the variable quantities of calcium monophosphate expressed as P_2O_5 shown in table 1.

Data regarding the soils used in this study are given in previous publications as follows: Nos. 188 to 392 (*8*, *15*); Nos. 6678 to 8736 (*12*); 9475 and B407 (*13*); 9804 and 9805 (*4*); B1086 (*2*); C799 to C805, C1478, and C1479 (*1*); C913, C1672, and C1673 (*3*); C2917 and C2918 (from the same location as Nos. 10083 and 10084, respectively), and C2929 and C2930 (from the same location as Nos. 10305 and 10306) (*4*). Soils C3175 and C3177 are described in a forthcoming publication.[3]

PRELIMINARY EXPERIMENTS

In preliminary work a few experiments were conducted with whole soils and with quartz sand. The results are given in table 1.

The results show that sodium selenite is highly toxic in quartz sand but relatively nontoxic in whole soils. Only 0.0014 gm. of selenium as selenite per pot was required to produce half injury in quartz sand; whereas in the Chester, Vernon, and Muskingum soils, 0.0100 gm of selenium produced no injury whatever, and in the Kirvin B soil even 0.0800 gm. had no effect on growth. In the Colby soil, however, 0 0068 to 0.0100 gm. of selenium was sufficient to reduce growth one-half. Quite different results were obtained in previous work with sodium selenate. The selanate was only a little less toxic in whole soils than in quartz sand. The average half-toxicity value for 14 whole soils was 0.0066 and for quartz sand in the same experiments, 0.0042. Although soils have no appreciable capacity for fixing the selenate ion, they evidently have a considerable capacity for fixing selenite

In none of these experiments was there any evidence of growth being stimulated by sodium selenite.

Seemingly sodium selenite is more toxic in quartz sand than sodium selenate. In these experiments 0.0014 gm. of selenium as selenite per pot produced half injury in quartz sand as compared with 0.0044 gm. of selenium as selenate, found in previous work (*7*). However, the toxicity of the selenate varies markedly with the sulfate supply (*7*, *10*). An experiment was therefore conducted to see whether selenite toxicity is likewise affected by the sulfate concentration. In this experiment Marquis wheat was grown in quartz sand, eight plants per pot; the sulfate supply was varied by substituting ammonium sulfate in the standard fertilizer. The quantity of monocalcium phosphate applied was equivalent to 0.20 gm. of phosphorus per pot. The results are shown in table 2.

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[3] ANDERSON, M S, KEYES, MARY G, and CROMER, GEORGE W SOLUBLE MATERIAL OF SOILS IN RELATION TO THEIR CLASSIFICATION AND GENERAL FERTILITY. U S Dept Agr Tech. Bul 813 [In press.]

TABLE 1—*Effect of different whole soils on toxicity of sodium selenite to millet*

EXPERIMENT 1 (MAY 24-JUNE 18)

Soil sample No	Kind of soil	Depth	CaCO₃ applied per pot	P₂O₅ applied per pot	Sodium selenite added per pot (expressed as Se)	Air dry yield per pot of plants above ground: Series A	Series B	Series C	Average	Se per pot required to reduce yield of plants by one half
		Inches	*Grams*	*Grams*	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*
	Quartz sand		0	0.20	0	4.3[illegible]	3.94	3.78	[illegible]	(1)
					.0050	.19	.15	.1[illegible]	.1[illegible]	
					.0100	.08	.04	.0[illegible]	0	
300	Chester loam	0-8	2.0	2.00	0	4.4[illegible]	4.83	4.1[illegible]	4.47	
					.0050	4.24	4.[illegible]	4.5[illegible]	4.4[illegible]	
					.0100	4.07	4.45	4.9[illegible]	4.49	
6719	Vernon fine sandy loam	0-10	0	.40	0	4.77	4.76	4.60	4.71	
					.0050	4.42	4.[illegible]0	4.43	4.4[illegible]	
					.0100	4.60	4.44	4.[illegible]0	4.3[illegible]	
6842	Colby silty clay loam	2-10	0	.20	0	5.97	7.57	7.88	7.11	0.0068
					.0050	4.14	4.2[illegible]	5.2[illegible]	4.[illegible]4	
					.0100	1.98	1.[illegible]8	2.11	1.90	

EXPERIMENT 2 (SEPT 1-24)

Soil sample No	Kind of soil	Depth	CaCO₃ applied per pot	P₂O₅ applied per pot	Sodium selenite added per pot (expressed as Se)	Series A	Series B	Series C	Average	Se per pot required to reduce yield of plants by one half
	Quartz sand		0	0.20	0	3.10	3.29	3.09	[illegible]	0.0014
					.0015	1.[illegible]	1.10	1.[illegible]	1.47	
					.0030	.31	.3[illegible]	.25	.30	
B407	Muskingum silt loam	0-[illegible]	2.0	.27	0	5.70	5.71	6.09	[illegible].83	
					.0050	5.[illegible]	5.[illegible]4	5.[illegible]5	[illegible]2	
					.0100	[illegible].64	5.[illegible]	[illegible].80	5.[illegible]0	
[illegible]	Kenyon fine sandy loam	12-24	2.0	2.6[illegible]	0	3.18	3.60	3.44	[illegible]41	
					.0400	3.[illegible]5	3.[illegible]2	3.12	3.[illegible]0	
					.0800	3.13	3.87	3.02	3.34	
[illegible]	do	0-12	0	.6[illegible]	0	[illegible].7[illegible]	3.78	3.44	3.[illegible]	
					.0200	3.3[illegible]	3.47	3.4[illegible]	3.42	
					.0400	3.2[illegible]	3.10	3.[illegible]	3.40	

EXPERIMENT 3 (OCT 12-NOV 13)

Soil sample No	Kind of soil	Depth	CaCO₃ applied per pot	P₂O₅ applied per pot	Sodium selenite added per pot (expressed as Se)	Series A	Series B	Series C	Average	Se per pot required to reduce yield of plants by one half
	Quartz sand		0	0.20	0	2.00	2.31	2.[illegible]3	2.31	0.0012
					.0015	.82	.9	.85	.82	
					.0040	.14	.1	.10	.13	
[illegible]	Colby silty clay loam	2-10	0	.20	0	3.80	4.02	4.[illegible]	4.17	.0100
					.0050	3.[illegible]	[illegible].91	3.[illegible]4	3.[illegible]	
					.0100	2.31	1.94	1.99	[illegible]08	

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[1] The half toxicity value is something less than 0.0026. The true value cannot be determined from the data because of the curvature of the toxicity curve and interpolation between distant points.

TABLE 2—*Influence of sulfate on toxicity of sodium selenite to wheat in quartz sand*

SO₄ applied per pot	Sodium selenite added per pot (expressed as Se)	Air dry yield per pot of plants above ground: Series A	Series B	Series C	Average	Average air dry yield of roots per pot	Se per pot required to reduce yield of plants above ground by one half	Se in air dry plants and limit of error of determination: Tops	Roots
Gram	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*	*P p m*	*P p m*
0.240	0	3.27	2.37	2.83	2.83	1.09	0.0032		
	.0020	2.11	2.13	2.00	2.08	.84		210±20	440±40
	.0040	.85	.89	.95	.90	.54		260±40	810±60
.120	0	3.03	3.19	3.42	3.21	1.15	.0024		
	.0020	1.84	1.88	1.88	1.87	.86		100±20	540±40
	.0040	.65	.95	1.10	.90	.60		370±40	830±50
.024	0	2.48	3.55	3.44	3.16	1.27	.0026		
	.0020	2.05	1.66	1.77	1.83	.84		160±20	320±40
	.0040	.64	.76	.83	.74	.49		400±40	820±70

In this experiment the selenite required for half injury is consta within the limits of experimental accuracy for fivefold and tenfo variations in the sulfate supply. Evidently selenite toxicity affected little or not at all by the sulfate concentration, at least long as the supply is sufficient for normal growth. Nevertheless, it possible that the injury produced by selenite, as well as by selenat may be due to an insufficiency of sulfur in the plant.

An experiment was also conducted to see whether toxicity of t selenite is influenced by the phosphate supply. This was necessar since in subsequent experiments the phosphate applications to diffe ent sand-soil mixtures were varied according to the estimated capa ties of the soils for phosphate fixation. Also, one might expect antagonism between phosphate and selenite. The results of t experiment, conducted with millet grown in quartz sand and in tw sand-soil mixtures, are shown in table 3.

The toxicity values for high and low phosphate applications are t same, within experimental error, in quartz sand and in the Marsh soil-sand mixture. In the Cecil soil-sand mixture it apparently tak more selenite to reduce the yield one-half with the lowest phospha application than it does with the two higher phosphate application It will be noted that the lowest phosphate application in the Ce soil-sand mixture was not sufficient for maximum growth in th mixture. It may be concluded that the toxicity value for sodiu selenite does not vary with the phosphate supply so long as this sufficient for maximum growth. Similar results were obtained pr viously with sodium selenate (*7*) and calcium arsenate (*6*).

SPECIFIC EFFECTS OF SOIL COLLOIDS ON SELENITE TOXICIT

The effect of different soil colloids on selenite toxicity was dete mined in sand-soil mixtures that contained sufficient soil to supply gm. of colloid per pot, equivalent to about 1 percent of the sand-so mixture. Millet was grown in all experiments except one in whic Marquis wheat was the crop plant. The results are shown in table The weight of the roots was recorded in order that the selenium a sorbed by the plant might be determined; but only the weight of to (the part of the plant above ground) was used in estimating t quantity of selenite that reduced growth one-half.

The half-toxicity values (selenium required to reduce the yie one-half) given in table 4 are those obtaining in quartz sand in sand-soil mixtures. By subtracting the sand value from that the sand-soil mixture, a half-toxicity value is obtained which repr sents the effect of 50 gm. of colloid per pot or of 1 percent of so colloid. These specific values, expressed as grams of selenium p pot, are shown in table 5. Data in tables 3 and 7 as well as in table were used in compiling this table. Values given for the Colb Marshall, Cecil, and Kirvin soils are averages of duplicate dete minations.

TABLE 3. *Influence of phosphate on toxicity of sodium selenite to millet in quartz sand and sand-soil mixtures*

Soil sample No.	Kind of soil mixed with quartz sand	Depth	P_2O_5 applied per pot	Sodium selenite applied per pot (expressed as Se)	Air-dry yield per pot of plants above ground					Average air-dry yield of roots per pot	Se per pot required to reduce yield of plants above ground by one half	Se in air-dry plants and limit of error of determination	
					Series A	Series B	Series C	Series D	Average			Tops	Roots
		Inches	*Grams*	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*	*Gram*	*P. p. m.*	*P. p. m.*
	Quartz sand only		0.20	0	0.93	1.10	1.39	1.21	1.16	0.18	0.0012	120±20	200±50
				.0013	.48	.52	.59	.57	.54	.10			
			.40	0	1.09	1.14	1.08	1.09	1.10	.18	.0017	110±20	420±100
				.0013	.58	.77	.71	.64	.68	.12			
5736	Marshall silt loam	0-13	.30	0	1.20		1.37	1.45	1.34	.15	.0041	50±10	240±50
				.0038	.63		.77	.79	.73	.10			
			.60	0		1.48	1.19		1.34	.15	.0045	30±10	80±30
				.0038	.69	.73	.87		.76	.09			
6977	Cecil sandy clay loam	0-6	.40	0	1.17	.80	1.00	1.18	1.04	.15	.0080	20±5	350±80
				.0092	.49	.44	.45	.41	.45	.09			
			.80	0	1.33	1.48	1.42	1.79	1.51	.22	.0062	80±20	670±140
				.0092	.38	.50	.29	.32	.37	.08			
			1.20	0	1.45	1.63	1.68	1.78	1.64	.22	.0058	70±10	690±140
				.0092	.31	.46	.32	.28	.34	.07			

TABLE 4.—*Effect of different soil colloids on toxicity of sodium selenite to plant growth in sand-soil mixtures*

[Millet grown except as otherwise indicated]

EXPERIMENT 6 (OCT 31-DEC 11)

Soil sample No	Kind of soil mixed with quartz sand	Depth	P_2O_5 applied per pot	Sodium selenite added per pot (expressed as Se)	Air dry yield per pot of plants above ground				Average air dry yield of roots per pot	Se per pot required to reduce yield of plants above ground by one-half	Se in air-dry plants and limit of error of determination	
					Series A	Series B	Series C	Average			Tops	Roots
		Inches	*Gram*	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*	*P p m*	*P p m*
	Quartz sand only		0.20	0	2.12	2.17	2.25	2.18		0.0013		
				.0015	.83	.87	.87	.86				
				.0040	.13	.13	.15	.14				
6678	Kirvin fine sandy loam	0-12	.40	0	2.59	2.19	2.60	2.46		.0183		
				.0150	1.22	1.33	2.08	1.54				
				.0300	.11	.08	.05	.08				
B407	Muskingum silt loam	0- 7	.20	0	3.02	3.05	2.88	2.98		.0060		
				.0050	1.43	1.73	1.78	1.65				
				.0100	1.15	.60	.79	.85				
				.0200	.02	.04	.03	.03				
C813	Manor loam	1- 8	.30	0	2.08	2.02	1.92	2.01		.0066		
				.0100	.52	.32	.63	.49				
				.0200	.05	.02	.02	.03				
6842	Colby silty clay loam	2-10	.20	0	1.05	1.04	1.33	1.14		.0038		
				.0025	.96	.60	.97	.84				
				.0050	.25	.33	.32	.30				
EXPERIMENT 7 (SEPT 9-OCT 5)												
	Quartz sand only		0.20	0	2.27	2.55	2.55	2.46	0.65	0.0015		
				.0013	1.50	1.25	1.30	1.35	.48		120± 20	700± 70
				.0026	.42	.43	.62	.49	.25		270± 70	540±130
8736	Marshall silt loam	0-13	.20	0	5.17	5.49	5.33	5.33	1.41	.0038		
				.0025	3.67	3.89	3.78	3.78	.91		30± 10	140± 40
				.0050	1.40	1.48	1.99	1.62	.46		80± 20	250± 70
6977	Cecil sandy clay loam	0- 6	.40	0	3.10	2.99	2.74	2.94	.60	.0092		
				.0070	2.60	1.66	1.66	1.97	.53		70± 20	440± 60
				.0140	.50	.23	.36	.36	.15		90± 20	330± 50
9475	Nacogdoches fine sandy loam	0- 8	.50	0	3.62	4.65	4.40	4.42	.93	.0108		
				.0100	2.27	1.89	2.67	2.28	.57		90± 20	290± 60
				.0200	.05	.03	.01	.03	.04		330±100	720±100
B1086	Spearfish silt loam	0-12	.30	0	4.78	4.94	5.09	4.94	1.23	.0049		
				.0030	3.78	3.30	3.96	3.68	1.05		40± 10	190± 30
				.0060	1.69	2.20	1.49	1.79	.48		110± 20	890± 70

EXPERIMENT 8 (SEPT. 25–NOV. 2)

....	Quartz sand only	..	0.20	0	4.81	4.40	4.03	4.41	0.88	0.0015		
				.0013	2.35	2.78	2.11	2.41	.65		70± 10	300± 50
				.0026	.93	1.00	1.03	.99	.38		70± 30	970± 90
6679	Kirvin fine sandy loam	12–24	.40	0	2.90	3.14	2.88	2.97	.57	.0065		
				.0100	.70	.66	.70	.69	.17		50± 10	490±100
				.0200	.03	.02	.03	.03	.01		900±300	
6719	Vernon fine sandy loam	0–10	.30	0	4.93	6.15	6.12	5.73	1.12	.0046		
				.0040	3.33	3.26	3.28	3.29	.73		80± 10	370± 50
				.0080	.32	.17	.31	.27	.11		90± 30	900±300
305	Stockton clay adobe	0–38	.20	0	5.37	5.33	5.37	5.36	1.07	.0041		
				.0015	4.32	4.77	4.19	4.43	.88		25± 5	080± 20
				.0030	3.25	3.55	3.44	3.41	.65		70± 10	360± 50
188	Orangeburg fine sandy loam	10–36	.40	0	3.07	4.40	4.02	3.83	.87	.0051		
				.0060	1.50	1.47	1.80	1.59	.42		80± 20	400± 80
				.0120	.13	.17	.25	.18	.10		270± 90	1200±350

EXPERIMENT 9 (OCT. 25–NOV. 29)

....	Quartz sand only	......	0.20	0	2.04	2.27	2.84	2.38	0.41	0.0018		
				.0013	1.42	1.50	1.53	1.48	.32		110±20	200± 50
				.0026	.68	.62	.72	.67	.19		170±50	600± 80
392	Hagerstown loam	8–30	.40	0	3.98	3.65	3.44	3.69	.54	.0032		
				.0040	1.45	1.30	1.41	1.39	.28		120±20	350±100
				.0080	.65	.45	.49	.53	.15		190±60	440±100
....	Nipe	15–26	.54	0	.33	.57	.32	.41	.07	------		
				.0100	.37	.34	.30	.34	.07		40±10	320± 90
				.0200	.01	.08	.08	.06	.04		180±60	360± 90
198	Sassafras silt loam	8–22	.40	0	3.48	3.56	3.49	3.51	.55	.0062		
				.0040	3.14	3.18	2.24	2.85	.47		40±10	140± 40
				.0080	.50	.88	.96	.78	.16		180±20	630±200
195	Clarksville silt loam	0–10	.30	0	3.74	2.76	3.48	3.33	.52	.0046		
				.0030	2.08	2.38	2.32	2.26	.43		90±10	230± 50
				.0060	1.01	1.32	1.33	1.22	.24		80±20	280±100

TABLE 4.—*Effect of different soil colloids on toxicity of sodium selenite to plant growth in sand-soil mixtures*—Continued

EXPERIMENT 10 (OCT. 16–NOV. 29)

Soil sample No.	Kind of soil mixed with quartz sand	Depth	P_2O_5 applied per pot	Sodium selenite added per pot (expressed as Se)	Air-dry yield per pot of plants above ground				Average air-dry yield of roots per pot	Se per pot required to reduce yield of plants above ground by one-half	Se in air-dry plants and limit of error of determination	
					Series A	Series B	Series C	Average			Tops	Roots
		Inches	*Gram*	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*	*P. p. m.*	*P. p. m.*
-------	Quartz sand only	- -	0.20	0	2.53	2.39	2.77	2.56	0.57	0.0017	----------	----------
				.0013	1.29	1.49	1.80	1.53	.41		44±10	490± 80
				.0026	.54	.62	.55	.57	.21		12± 5	480±160
C804	Hagerstown silt loam	3–12	.40	0	3.75	3.60	3.15	3.50	.61	.0050	----------	----------
				.0040	2.53	1.75	2.22	2.17	.43		11± 4	80± 15
				.0080	.60	.52	.47	.53	.14		50±10	----------
C805	do	12–33	.40	0	3.08	3.00	3.01	3 03	.49	.0040	----------	----------
				.0040	1.47	1.33	1.60	1.47	.35		45±10	----------
				.0080	.13	.25	.20	.19	.12		----------	680±150
C799	Hagerstown silty clay loam	2– 8	.40	0	4.02	3.56	4.25	3.94	.63	.0068	----------	----------
				.0040	3 45	2.78	3.41	3.21	.59		15± 5	80± 15
				.0080	1 38	1.03	1.81	1.41	.29		24± 5	170± 30
C800	do	8–14	.40	0	3.72	3.63	3.56	3.64	.62	.0042	----------	----------
				.0040	1.96	1.73	1.95	1.89	.41		32± 5	80± 15
				.0080	.43	.23	.37	.34	.11		60±20	440±100
C1672	Chester loam	10–34	.40	0	2 32	2 14	1.98	2.15	.45	.0067	----------	----------
				.0040	1.91	1.46	1.70	1.69	.36		30± 5	140± 30
				.0080	83	.72	.81	.79	.20		12± 4	200± 40
C1673	do	34–60	.40	0	1 83	1.72	1.47	1.67	.32	.0070	----------	----------
				.0040	1 58	1.55	1.33	1.49	.33		35±10	100± 20
				.0080	.61	.60	.64	.62	.15		50±10	340± 60

EXPERIMENT 11 (FEB 28 APR 1)

	Quartz sand only		0 20	0	3 80	3 32	3 36	3 49	0 60	0 0022		
				0013	2 55	2 52	2 85	2 64	49		20±5	170± 30
				0026	1 44	1 25	1 51	1 40	32		30±5	130± 40
C2917	Carrington loam	3 13	25	0	8 68	8 70	9 05	8 81	1 71	0064		
				0035	5 92	6 40	6 44	6 25	1 12		8±2	45± 10
				0070	3 72	4 67	3 86	4 08	56		30±5	270± 30
C2918	do	13 22	25	0	6 90	7 60	7 36	7 29	1 13	0060		
				0035	5 94	5 83	5 72	5 83	1 29		8±2	18± 6
				0070	2 25	3 22	2 82	2 76	46		35±5	110± 20
9804	Columbiana clay	0-10	40	0	6 88	6 78	6 43	6 70	1 45	0059		
				0045	4 07	4 18	4 18	4 14	55		20±5	80± 20
				0090	1 33	1 36	2 11	1 60	37		20±5	720±100
9805	do	10-25	40	0	6 30	5 58	6 12	6 00	1 08	0057		
				0045	3 58	3 73	3 75	3 69	88		18±3	270± 40
				0090	1 25	1 13	90	1 09	27		25±5	1 460±120
C2929	Barnes loam	0- 9	20	0	7 18	6 80	6 37	6 78	89	0042		
				0025	5 30	5 96	3 40	4 89	69		6±2	40± 10
				0050	2 76	2 13	3 39	2 76	47		15±3	50± 10
C2930	do	9-17	20	0	7 60	6 92	7 31	7 28	1 22	0044		
				0025	5 66	5 95	4 15	5 25	80		7±2	40± 10
				0050	2 81	3 93	2 58	3 11	41		15±3	40± 10

EXPERIMENT 12 (JAN 31 MAR 11

	Quartz sand only		0 20	0	3 50	2 93	3 45	3 29	0 60	0 0013		
				0013	1 30	1 67	1 79	1 59	41		15± 5	370± 40
				0026	70	65	69	68	21		50±10	620±120
C3175	Russell silt loam	2 6	25	0	3 40	4 33	3 62	3 78	79	0050		
				0040	2 38	2 12		2 25	49		12± 5	360±100
				0080	82	75	6	74	24		20±10	630±150
C3177	do	13 23	25	0	3 94	3 82	3 10	3 62	70	0049		
				0040	1 96	1 99	2 57	2 18	55		12± 3	30± 10
				0080	75	48	57	60	19		50±10	120± 40
C1478	Lebanon silt loam	0- 6	25	0	4 38	3 54		3 96	71	0072		
				0040	3 23	3 68		3 45	72		10± 3	40± 10
				0080	2 02	1 18		1 60	36		20± 5	110± 30
C1479	do	6 11	25	0	2 33	2 04	2 34	2 24	52	0048		
				0040	1 36	1 25	1 30	1 30	34		15± 5	80± 20
				0080	48	45	40	44	18		60±15	230± 60

TABLE 4.—*Effect of different soil colloids on toxicity of sodium selenite to plant growth in sand-soil mixtures*—Continued

EXPERIMENT 13 (DEC. 9–JAN. 26, PLANTED TO WHEAT)

Soil sample No.	Kind of soil mixed with quartz sand	Depth	P_2O_5 applied per pot	Sodium selenite added per pot (expressed as Se)	Air-dry yield per pot of plants above ground				Average air-dry yield of roots per pot	Se per pot required to reduce yield of plants above ground by one-half	Se in air-dry plants and limit of error of determination	
					Series A	Series B	Series C	Average			Tops	Roots
		Inches	*Gram*	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*	*P. p. m.*	*P. p. m.*
----------	Quartz sand only	----------	0.20	0	2.00	2.12	1.61	1.91	0.39	0.0031	----------	----------
				.0020	1.30	1.31	1.08	1.23	.34		160± 30	580± 60
				.0040	.73	.76	.74	.74	.28		450± 50	660±100
B407	Muskingum silt loam	0– 7	.20	0	2.12	1.87	1.85	1.95	.39	.0150	----------	----------
				.0060	1.57	1.92	1.61	1.70	.34		80± 20	300±100
				.0120	1.15	1.21	1.31	1.22	.31		140± 30	540±100
6842	Colby silty clay loam	2–10	.20	0	1.93	1.95	2.04	1.97	.38	.0076	----------	----------
				.0060	1.39	1.37	1.10	1.29	.33		130± 20	600±100
				.0120	.21	.21	.31	.24	.11		700±100	1500±300
9475	Nacogdoches fine sandy loam	0– 8	.40	0	2.19	2.15	1.88	2.07	.40	.0226	----------	----------
				.0100	1.88	2.07	2.15	2.03	.40		25± 5	330±100
				.0200	1.27	1.37	1.11	1.25	.33		110± 30	500±100

EXPERIMENT 14 (FEB 16–MAR 23)

				0	2 75	2 96	2 70	2 80	0 64			
	Quartz sand only		0 20	0013	1 36	1 20	1 23	1 26	37	0 0012	40± 5	250± 50
				0026	61	62	51	58	20		35± 5	500±100
				0	4 35	5 15	4 68	4 73	90			
300	Chester loam (50 gm colloid)	0– 8	30	0050	3 62	3 05	2 64	3 10	65	0077	8± 1	20± 5
				0150	17	59	37	38	12		35± 10	800±150
				0	4 95	4 95	4 90	4 93	97			
300	Chester loam (20 gm colloid)	0 8	24	0020	46	35	84	55	14	0011	6± 3	70± 20
				0060	02	06	06	05	04			80± 40
				0	4 49	4 08	3 50	4 02	66			
6678	Kirvin fine sandy loam (60 gm colloid)	0–12	40	0120	2 92	2 79	2 11	2 61	41	0168	10± 2	150± 30
				0240	1 25	1 13	83	1 07	21		18± 6	300± 80
				0	2 60	3 24	3 48	3 11	52			
6678	Kirvin fine sandy loam (20 gm colloid)	0 12	28	0040	3 37	2 78	2 48	2 88	52	0096	6± 2	160± 30
				0080	1 50	2 12	2 24	1 95	39		35± 10	50± 10
				0	3 60	3 93	3 92	3 82	85			
9805	Columbiana clay (75 gm colloid)	10 25	50	0150	44	43	38	42	1	0084	200± 40	750±100
				0300	02	03	03	03			400±100	
				0	2 10	1 68	2 83	2 20				
6842	Colby silty clay loam (50 gm colloid)	2 10	20	0030	1 28	1 15	1 35	1 23		0035	20± 4	
				0060	29	52	30	37			45± 5	
				0	1 90	1 78	2. 12	1 93		0055		
6842	Colby silty clay loam* (150 gm colloid)	2 10	20	0040	84	1 40	1 43	1 22			10± 2	

Plants affected with root rot.

TABLE 5.—*Specific effects of soil colloids on selenite toxicity as related to chemical composition*

Soil sample No	Kind of soil	Depth	Effect of 50 gm soil colloid on half-toxicity value (expressed as Se per pot)	Percentage of iron (Fe_2O_3) in colloidal material [1]	Molecular ratio of SiO_2 to Al_2O_3+ Fe_2O_3 in colloidal material [2]
		Inches	*Gram*	*Percent*	
392	Hagerstown loam	8–30	0 0014	11 31	1 89
C2929	Barnes loam	0– 9	0020	9 96	3 05
C2930	do	9–17	0022	10 93	3 00
C805	Hagerstown silt loam	12–33	0023	9 62	2 03
6842	Colby silty clay loam	2–10	.0024	7 10	3 41
C800	Hagerstown silty clay loam	8–14	0025	10 80	1 86
8736	Marshall silt loam	0 13	0026	9 10	2 91
305	Stockton clay adobe	0 38	0026	10 57	2 87
195	Clarksville silt loam	0 10	0028	10 05	2 18
6719	Vernon fine sandy loam	0 10	0031	10 70	2 50
C804	Hagerstown silt loam	3–12	0033	9 50	2 10
B1086	Spearfish silt loam	0–12	0034	6 40	2 84
C1479	Lebanon silt loam	6–11	0035	9 60	2 56
9805	Columbiana clay	10–25	0035	16 15	81
188	Orangeburg fine sandy loam	10–36	0036	10 06	1 71
C3177	Russell silt loam	13–23	0036	12 41	2 36
C3175	do	2– 8	0037	11 00	2 43
9804	Columbiana clay	0–10	0037	15 36	81
C2918	Carrington loam	13–22	0038	10 07	2 38
C2917	do	3 13	0042	10 09	2 39
198	Sassafras silt loam	8–22	0044	12 73	1 89
B407	Muskingum silt loam	0– 7	.0047	9 97	2 26
C1672	Chester loam	10–34	0050	17 56	1 74
6379	Kirvin fine sandy loam	12–24	0051	14 37	1 74
C799	Hagerstown silty clay loam	2 8	0051	11 52	1 70
C813	Manor loam	1– 8	0053	10 33	1 81
C1673	Chester loam	34 60	0053	20 21	1 53
C1478	Lebanon silt loam	0– 6	0059	8 80	2 74
6977	Cecil sandy clay loam	0– 6	0064	14 57	1 34
300	Chester loam	0 8	0065	15 93	1 54
9475	Nacogdoches fine sandy loam	0 8	0093	28 05	1 07
6678	Kirvin fine sandy loam	0 12	0170	14 71	2 02

[1] Coefficient of correlation of selenium values with percentage of iron (Fe_2O_3), +0 51

[2] Coefficient of correlation of selenium values with molecular ratio, $\frac{SiO_2}{Al_2O+Fe_2O_3}$, −0 32

The accuracy of the half-toxicity values shown in table 5 cannot be calculated exactly, but probably most values are accurate within 10 to 20 percent of the values given. This is indicated by the agreement of duplicate determinations appearing in tables 3, 4, and 7. The duplicate half-toxicity values of the soil colloids are as follows: Colby, 0.0025 and 0.0023; Marshall, 0.0023 and 0.0029; Cecil, 0.0077 and 0.0050; and the Kirvin 12- to 24-inch layer, 0.0050 and 0.0051. There are also 14 determinations of selenite toxicity in pure quartz sand. These are not strictly duplicate determinations, since different shipments of sand varied in the impurities present, but they give some idea of the experimental error. The extremes of the 14 determinations are 0.0010 and 0.0022; the average value is 0.0014, and the standard deviation ±0.0003.

The values given in table 5 show that the soil colloids vary widely in their effect on sodium selenite. The highest value is more than 10 times the lowest. This is in strong contrast with the uniform absence of any effect on sodium selenate. However, in previous work a somewhat greater variation was found in the effect of different colloids on calcium arsenate (*6*).

The values given in table 5 should fairly represent the comparative effects of the different soil colloids on selenite, since they were obtained by measuring the effects of equal quantities of material under similar conditions. It seems, however, that these values representing the effect of 1 percent of colloid cannot safely be used for calculating the effects of the whole soils on selenite. When the values are multiplied by the percentage of colloid in the soil, the results in some instances are quite far from the actual effect of the whole soils. This is indicated by the results of experiment 14 (table 4) in which the effects of increasing quantities of colloid are compared.

It will be seen that the effect of the colloid on selenite increases with the quantity of colloid applied, but the results are not consistent. In the case of the Colby and Kirvin soil-sand mixtures, increasing the colloid by three times increases the effect on selenite not by three times but by approximately two times. The result with 20 gm. of Chester colloid is anomalous but apparently is not due to experimental error. In previous work with calcium arsenate the effect of the colloid increased in direct proportion to the quantity applied, up to applications of 100 or 150 gm. of colloid, although it was presumed that the relation would not hold for much larger quantities of colloid. The poorer results obtained with selenite might be due to the fact that sodium selenite was applied in solution to the top of the pot; whereas the calcium arsenate was mixed with the whole volume of the sand-soil mixture.

The specific effects of the colloids bear little relation to the broad groups of soils established by soil classification. Some of the data, such as the comparative effects of topsoil and subsoil colloids, indicate that the effect of a colloid may be associated with a minor characteristic. Values given in table 5 for two layers of the same soil are brought together in table 6.

TABLE 6.—*Effects of colloids in two different layers of the same soil type on half-toxicity values*

Kind of soil	Depth of soil sample		Effect of soil colloid on half-toxicity value (expressed as Se per pot)	
	Upper	Lower	Upper	Lower
	Inches	*Inches*	*Gram*	*Gram*
Barnes, Nos. C2929 and C2930	0-9	9-17	0.0020	0.0022
Carrington, Nos. C2917 and C2918	3-13	13-22	.0042	.0038
Chester, Nos. C1672 and C1673	10-34	34-60	.0050	.0053
Columbiana, Nos. C9804 and C9805	0-10	10-25	.0037	.0035
Hagerstown, Nos. C799 and C800	2-8	8-14	.0051	.0025
Hagerstown, Nos. C804 and C805	3-12	12-33	.0033	.0023
Kirvin, Nos. 6678 and 6679	0-12	12-24	.0170	.0051
Lebanon, Nos. C1478 and C1479	0-6	6-11	.0059	.0035
Russell, Nos. C3175 and C3177	2-8	13-23	.0037	.0036

In five soils the colloidal material of the deeper soil layer had almost exactly the same effect on selenite toxicity as the colloid in the layer nearer the surface, but in four other soils the upper soil colloid was considerably more effective than the lower. It was thought possible that the greater effectiveness of the upper colloid obtaining in some cases might be due to organic matter. Several experiments were conducted to see if this was correct.

TABLE 7.—*Effects of organic matter, water content, and iron gels on toxicity of sodium selenite*[1]

EXPERIMENT 15 (NOV. 12–DEC. 21)

Soil or material added to quartz sand	Water content of pot	P_2O_5 applied per pot	Sodium selenite per pot (expressed as Se)	Air-dry yield per pot of plants above ground				Average air-dry yield of roots per pot	Se per pot required to reduce yield of plants above ground by one half	Se in air-dry plants and limit of error of determination	
				Series A	Series B	Series C	Average			Tops	Roots
	Percent	*Gram*	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*	*P. p. m.*	*P. p. m.*
Quartz sand only		0.20	0	1.98	1.93	2.00	1.97	0.35	0.0012	90±20	450±100
			.0013	.65	1.05	1.00	.90	.19			
Peat		.20	0	2.30	2.48		2.39	.36	.0027	9± 3	210± 50
			.0013	1.84	1.78		1.81	.28			
Peat and $CaCO_3$		.20	0	2.43	3.13		2.78	.42	.0018	40±10	150± 40
			.0013	1.73	1.82		1.78	.27			
Activated charcoal		.20	0	2.37	2.55		2.46	.40	.0018	60±10	240± 80
			.0013	1.65	1.53		1.59	.31			
Humus		.20	0	2.40	2.68		2.54	.39	.0023	20± 5	170± 30
			.0013	1.70	1.95		1.83	.30			
Kirvin soil, No. 6679 only		.40	0	1.77	1.08	1.38	1.41	.20	.0063	100±20	640±130
			.0070	.56	.67	.68	.64	.13			
Kirvin soil, No. 6679+peat		.40	0	2.84	3.59		3.22	.47	.0058	20± 5	70± 20
			.0070	1.24	1.27		1.26	.21			
Kirvin soil, No. 6679+peat+$CaCO_3$		.40	0	3.11	3.24		3.18	.38	.0058	30± 5	90± 30
			.0070	1.17	1.36		1.27	.19			
Kirvin soil, No. 6679+activated charcoal		.40	0	2.43	2.37		2.40	.32	.0045	90±20	560±100
			.0070	.40	.70		.55	.16			
Kirvin soil, No. 6679+humus		.40	0	1.54	1.30		1.42	.23	.0085	90±20	580±100
			.0070	.78	.90		.84	.16			

RICE GROWN INSTEAD OF MILLET EXPERIMENT 16 (FEB 21 APR 4)											
Quartz sand only	15	0.20	0	4.02	3.65	4.09	3.93	1.50			
			.0015	1.76	1.92	2.35	2.01	.46	0.0015	70±10	150±30
			.0030	.75	.97	.90	.87	.40		90±20	680±80
	29	.20	0	5.47	5.81	5.05	5.44	2.14			
			.0015	3.27	4.06	4.25	3.86	1.24	.0025	25± 5	130±30
			.0030	1.89	2.28	2.55	2.24	.75		130±20	350±50
Muskingum soil, No B407	15	.20	0	5.45	4.87	5.38	5.23	1.79			
			.0050	4.20	3.98	4.10	4.06	1.15	.0079	25± 5	120±30
			.0100	1.60	1.22	2.16	1.66	.50		50±10	120±20
	29	.20	0	5.43	5.98	5.56	5.66	2.10			
			.0050	4.27	4.95	4.05	4.42	1.44	.0083	15± 3	35±10
			.0100	1.16	2.98	1.99	2.04	.58		40±10	140±30
Hyperhumus	15	.20	0	4.37	4.62	3.80	4.26	1.65			
			.0020	1.92	1.72	2.25	1.96	.62	.0019	100±20	30± 5
			.0040	.57	.72	.88	.72	.28		80±20	180±40
	29	.20	0	4.97	5.01	5.25	5.08	1.79			
			.0020	2.85	3.07	2.52	2.81	.85	.0026	40±10	100±20
			.0040	1.90	2.40	2.06	2.12	.68		60±10	
Muskingum soil, No B407+Hyperhumus	15	.20	0	5.77	5.96	5.07	5.60	1.93			
			.0050	4.06	4.32	4.31	4.23	1.12	.0087	12± 3	60±20
			.0100	2.78	1.31	2.79	2.29	.59		20± 5	90±20
	29	.20	0	5.32	5.75	5.39	5.49	1.74			
			.0050	4.52	4.36	4.16	4.35	1.29	.0085	15± 3	260±30
			.0100	2.15	2.77	1.19	2.04	.57		40±10	70±15
EXPERIMENT 17 (MAR 1 APR 4)											
Quartz sand only		0.20	0	3.52	2.80	3.98	3.33	0.76			
			.0013	1.00	.96	1.22	1.06	.40	0.0010	60±15	400±80
Iron gel, dried		50	0	6.22				1.40			
			.0100	2.32				.80	.0080	30± 5	600±150
			.0200	.47				.21		60±10	950±250
			.0300	.07				.02		140±70	500±250
EXPERIMENT 18 (DEC 27 FEB 10)											
Quartz sand only		0.20	0	1.34	1.50	1.65	1.50	0.27			
			.0015	.65	.80	.98	.51	.19	0.0017	40±10	430± 80
			.0030	.27	.36	.34	.32	.10		150±20	1 100±200
Iron gel, moist		50	0	1.30				.23			
			.0060	1.03				.22			15±10
			.0120	.54				.14			7± 7
			.0240	1.35				.26	1094	10± 5	200±100
			.0480	.70				.17		30±15	120± 60
			.0960	.80				.23		70±15	430± 80
			.1920	.14				.06		200±70	1 300±400

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[1] Soils added at rate of 50 gm per pot, organic materials and iron gels at rate of 10 gm per pot. Millet grown except as otherwise indicated

EXPERIMENTS TO EXPLAIN SPECIFIC EFFECTS OF DIFFERENT SOIL COLLOIDS

In experiment 15 (table 7), various organic materials were applied in conjunction with the Kirvin 12- to 24-inch material to see if the two materials together would have an effect approaching that of the Kirvin surface layer. The Kirvin subsoil was added to quartz sand at a rate to supply 50 gm. of colloid per pot; the organic materials were added at the rate of 10 gm. per pot. The peat was a reed peat of pH 4.84; the "peat+$CaCO_3$" was the same peat limed to pH 6.81. The activated charcoal was a commercial product rendered practically neutral by washing with distilled water. The so-called humus was the finest, more decomposed forest litter found on top of Chester soil. It had a pH value of 3.89. Millet was the crop plant.

The organic materials alone, that is, mixed with quartz sand, all had slight capacities for reducing the toxicity of sodium selenite. Added to the mixture of sand and Kirvin soil, only the humus increased the capacity of the mixture for rendering selenite nontoxic, but the half-toxicity value characteristic of the whole mixture (sand+Kirvin soil+humus) was not significantly greater than that of the separate constituents. The results of this experiment do not support the idea that the toxicity values of some A horizons are higher than those of B horizons owing to the presence of more organic matter.

It was thought possible, however, that some kind of organic matter might affect toxicity values by creating reducing conditions. An experiment was conducted in which exaggerated reducing conditions were produced by maintaining an excessive water content in the sand-soil mixtures and by maintaining an excessive water content in conjunction with added organic matter Robinson (*14*) has shown that when soils are kept under submerged conditions considerable iron is rendered soluble, particularly in the case of soils high in organic matter. If soluble iron were produced in the sand-soil mixture, this might combine with the selenite and render it less toxic.

Experiment 16, in which excess water was kept in some of the pots, was conducted with rice, since this plant grows well in soil with either a normal or excessive water content. In this experiment the standard fertilizer shown on page 559 was applied; 7 rice plants were grown per pot; 10 gm. of Hyperhumus was applied per pot, and the Muskingum soil was applied at the rate of 50 gm. of colloid per pot. The Hyperhumus is a commercial peat of pH 5, used currently for soil improvement. All pots were kept at a water content of 15 percent until the plants formed their third leaf. The water content of half the pots was then raised to 29 percent, which was sufficient to more than saturate the sand and the sand-soil mixtures. The results of experiment 16 are shown in table 7.

The sodium selenite was somewhat less toxic in quartz sand at the higher water content than at the lower; that is, the half-toxicity value of the sand was greater at the high water content. Whether the capacities of the soil and peat for reducing selenite toxicity were affected by the water content can be seen when the half-toxicity values of quartz sand are subtracted from those of the sand-soil mixtures. Under low and high water content the Muskingum soil had half-toxicity values of 0.0064 gm. and 0.0058 gm., the Hyperhumus had half-toxicity values of 0.0004 gm. and 0.0003 gm., and the mixture of Muskingum soil and Hyperhumus had half-toxicity values of 0.0072 gm. and 0.0060 gm. It is also to be noted that the half-toxicity values of the mixture of Muskingum soil and Hyperhumus are almost exactly the sum of the half-toxicity values of the separate constituents. Evidently reducing conditions induced by saturation of the sand-soil mixtures had no effect on the capacity of Muskingum soil colloids for rendering sodium selenite less toxic, even when considerable organic matter was present.

Before experiment 16 (table 7) was carried out, a similar experiment had been conducted with a moss peat of pH 5 instead of Hyperhumus. Seed was planted on January 2, but growth was so weak and spindling, presumably owing to insufficient sunlight, that the plants were cut on January 25. The air-dry yields of pots receiving no selenium were only about 0.10 gm. per pot. It was assumed that this experiment was a complete failure; hence seed for experiment 16 (table 7) was planted on February 21. It is of interest to note that the slight yields of this seemingly abortive experiment gave half-toxicity values similar to those of experiment 16 just discussed. Under low and high water content the half-toxicity values were as follows: For quartz sand 0.0015 and 0.0019 gm.; for the Muskingum soil, 0.0075 and 0.0071 gm.; for the peat, 0.0005 and 0.0021 gm.; and for the mixture of peat and Muskingum soil, 0.0070 and 0.0081 gm.

Naturally it was thought that the effect of the colloidal materials on selenite was at least partly due to the iron content, since work of Williams and Byers (*16*) had indicated that soils may contain an insoluble basic ferric selenite. Experiments were therefore conducted with artificial iron gels; not that positive results would necessarily prove the correctness of the idea, but that negative results would practically disprove it. The results of experiments 17 and 18 with a dry and a moist iron gel are shown in table 7. The gels were prepared from ferric chloride and sodium hydroxide. The precipitates were washed by decantation until they began to disperse. The moist gel was then dried on a steam bath. About 10 gm. of the dry gel and a quantity of moist gel equivalent to the 10 gm. of dry gel were applied per pot. It was necessary to apply a large amount of phosphate to the pots receiving iron gels in order to supply the plants with phosphoric acid. The results of experiment 18 indicate that the quantity of phosphate applied to pots containing the moist gel was insufficient. The variable yields with selenite applications less than 0.0960 gm. of selenium per pot were presumably due to variations in available phosphate. It was difficult to mix the phosphate evenly with the moist mixture of sand and iron gel. The value of about 0.1 gm. of selenium required for reducing the yield one-half in the presence of the moist gel is probably only approximate, but it is evidently many times greater than the 0.0070 value characterizing the dry gel. The effect of iron gels on sodium selenite obviously varies greatly with the condition of the gel and may be very considerable.

SPECIFIC EFFECTS OF SOIL COLLOIDS AS RELATED TO THEIR CHEMICAL COMPOSITION

The figures shown in column 4 of table 5 are strictly the results of the soil applications but are assumed to represent the effects of the 50 gm. of colloidal material present. Presumably the values are specific values for the soil colloids and may be regarded as showing that the selenite, expressed as grams of selenium per pot, was rendered unavailable or nontoxic by 50 gm. of colloid. These values are significantly but imperfectly related to the ultimate chemical composition of the soil colloids. As shown at the bottom of table 5, the effects of the colloids on selenite are more closely related to the percentages of iron present than to the silica-sesquioxide ratios of the colloids. The molecular ratios of iron to silica plus alumina were calculated and found to be related to the selenite values in about the same degree as the simple percentages of iron. The coefficient of correlation was +0.44.

These results are similar to those obtained in a previous study dealing with the effects of soil colloids on the toxicity of calcium arsenate (*6*). The coefficient of correlation between the arsenate values and percentages of iron was +0.59, the same, within limits of error, as the correlation between selenite and iron. It would seem that the arsenate and selenite values should be quite closely correlated, but apparently they are not. Twelve of the soils tested in this study of sodium selenite were used in the work on calcium arsenate. The effects of these soils on selenite toxicity are related to the effects on arsenate toxicity only to the degree expressed by the coefficient 0.47.

The fact that toxicity values of sodium selenite are not more closely related to the percentages of iron than by coefficient 0.51 may be explained on the ground that it is reactive iron rather than total iron which is effective in rendering the selenite nontoxic. Unfortunately, methods are not available for determining the reactivity of iron in the soil colloids. But presumably the soil colloids may contain iron in forms that vary widely in their reactivity with selenite, just as did the moist and dried iron gels previously mentioned. It is known that a varying proportion of the iron is present as silicate and that the free iron oxide varies in crystalline structure and degree of hydration. Possibly each colloid contains not simple reactive and nonreactive iron but iron in various degrees of reactivity, just as artificial catalysts contain atoms of varying reactivity. Such a condition could account for the significant, but not close, correlation between the specific effects of the colloids on arsenate and selenite.

Another factor affecting the toxicity values may be microbiological activity. There was no control of this in the experiments reported. According to Maassen (*11*) there are several micro-organisms capable of reducing selenium salts to volatile organic compounds; presumably sodium selenite could be reduced to metallic selenium in the soil, since Hurd-Karrer has reported the presence of metallic selenium in wheat roots (*10*). Hurd-Karrer (*9*) found elementary selenium nontoxic. The toxicities of volatile selenium compounds in the soil have not been determined, but presumably they are different from that of sodium selenite. The formation of such compounds, therefore, would probably affect the toxicity values.

The fact that during an experiment there was usually a characteristic selenium odor in the greenhouse indicates that there was some microbiological action on the selenite applied. But whether it was of such a character or magnitude as to affect the half-toxicity values appreciably is uncertain. Evidently it was not the chief factor affecting the values. If it had been, the correlation between the iron content of the colloids and their effects on selenite would hardly have been as high as +0.51.

SELENIUM ABSORBED BY THE PLANTS

The quantities of selenium absorbed by the plants were determined by separate analyses made of the roots and tops by the method described by Williams and Lakin (*17*). The limits of accuracy of the selenium determinations are shown by the figures following the ± sign. A value of 40±5 p. p. m. indicates that the plant substance contained at least 35 p. p. m. of selenium and not more than 45 p. p. m Large values for the limit of error are due to the small quantities of plant substance available for analysis. Because of the large allowance that must be made for the limits of analytical error in many instances, conclusions cannot be drawn regarding the comparative selenium content of many individual samples. But conclusions based on average figures of several samples should be reliable. The following generalizations can be made with considerable certainty.

When plants are grown with selenium applied as sodium selenite the roots contain a much higher percentage of selenium than the tops. There are no exceptions to this generalization in the millet experiments. The percentage of selenium in millet roots averages 8.6 times the percentage in the tops for 107 samples grown in experiments reported here. For 23 samples of rice the percentage of selenium in the roots averages 3.9 times that in the tops, and in 14 samples of wheat the average figure for the roots is 3.1 times that for the tops. The wide variations in the relative amounts of selenium in the roots and tops in different samples indicate that no fixed proportion should be expected in the percentages in the 2 parts of the plant.

With selenium applied as sodium selenate the opposite condition obtains; the concentration of selenium in the tops exceeds that in the roots. This was not established in the writers' previous experiments with millet, since only the tops were analyzed. Hurd-Karrer (*10*), however, has shown that it holds for wheat, and the writers have found that it holds for radishes. In an experiment with the Manor soil, in which four lots of radishes were grown with additions of sodium selenate, the selenium content of the radish roots ranged from 350 to 690 p. p. m., while the tops contained from 760 to 1,800 p. p. m.

The concentration of selenium in the whole plant (the weighted average of the percentages of selenium in roots and tops) tends to increase in direct proportion to the quantity of sodium selenite applied. This is apparent on comparing the selenium content of plants growing with single and double applications of sodium selenite. Thirty-four such comparisons for millet can be made from data given in tables 4 and 7. The plants grown with the double applications of sodium selenite contain on an average 2.7 times as much selenium as plants grown with the single applications. Many individual cases vary widely from this relation, as shown by the fact that the figure 2.7 is subject to a standard deviation of ± 1.2.

If it is assumed that selenium in the plant varies directly with the quantity of selenium applied, the selenium content of plants at half injury can be calculated from the data given in tables 4 and 7. When this is done it appears that plants grown in the sand-soil mixtures usually contain a smaller concentration of selenium at half injury than plants grown in quartz sand. This conclusion is based on 32 comparisons for millet and 6 for rice; the only exceptions to the generalization are 6 of the comparisons for millet. The average figure for the 32 millet comparisons is only 0.72 as much selenium in the plants grown in sand-soil mixtures as in plants grown in pure sand. The average figure for the 6 comparisons involving rice is only 0.48 as much selenium in the plants grown in sand-soil mixtures. This difference in the selenium content of plants grown in sand-soil mixtures and in pure sand is probably connected with the different growth conditions in the 2 mediums, but it is not evident what the determining condition is. The fact that the selenium content of plants grown in sand-soil mixtures is less than that of sand-grown plants at the same degree of injury indicates that the soil application affects the toxicity of selenium within the plant.

COMPARATIVE EFFECTS OF SODIUM SELENITE ON WHEAT, RICE, AND MILLET

The comparative effects of sodium selenite on wheat, rice, and millet may be judged from figures given in previous tables for the quantities of selenite required to produce half injury. These figures are brought together in table 8. The half-toxicity value for millet in quartz sand is the average of 14 determinations, and the value for wheat in quartz sand is the average of 2 almost identical determinations shown in table 2 and in table 4, experiment 13.

In pure quartz sand, sodium selenite is equally toxic for rice and millet but only half as toxic for wheat; that is, it requires twice as much selenite to reduce the yield of wheat one-half as it does to reduce the yield of millet one-half. A similar difference between millet and wheat was found in previous work with sodium selenate (7). The growth of millet in quartz sand was reduced one-half by 0.0044 gm. of selenium as selenate per pot, whereas 0.0072 gm. of selenium was required to produce the same effect on wheat (7). Apparently the concentration of selenite or selenate required to injure wheat is greater than that required to injure millet, not because wheat is more resistant to absorption of the ions but because it is less injured by the ions after they are absorbed. This is shown by the analytical data; the wheat plants are considerably higher in selenium than the millet plants.

TABLE 8.—*Effects of quartz sand and soil colloids on sodium selenite required for half injury of millet, rice, and wheat*

Material affecting selenite toxicity	Effect of material on selenium per pot required to produce half injury in—		
	Millet	Rice	Wheat
	Gram	*Gram*	*Gram*
Quartz sand only	0.0014	0.0015	0.0032
50 gm Muskingum soil colloid	.0047	.0064	.0119
50 gm Colby soil colloid	.0024		.0045
50 gm. Nacogdoches soil colloid	.0093		.0195

It is significant that the values representing the effects of the soil colloids on selenite toxicity are twice as great when measured by wheat as when measured by millet. As previously mentioned, the effect of the soil is obtained by subtracting the quantity of selenium required for half injury in the sand from the selenium required for half injury in the sand-soil mixtures. The blank for the sand is twice as great for wheat as for millet; but if the colloidal material combined with the selenite to form a complex wholly unavailable to plants, the value representing the effect of the colloid on the selenite required for half injury should be no more for wheat than for millet. However, if the selenite-colloid combination is slightly available to plants, the effect of the colloid on sodium selenite might be expected to be twice as large when measured by wheat as when measured by millet. As compared with millet, wheat would require twice as much of the slightly available compound for half injury, just as it required twice as much of the wholly available selenite in quartz sand.

DIFFERENCE BETWEEN THE EFFECTS OF SODIUM SELENITE AND SODIUM SELENATE

In the two experiments with wheat shown in tables 2 and 4, the plants injured by selenite were normal in appearance although they tended to wilt more readily than normal plants. In the previous work with sodium selenate the ivory-white chlorosis considered typical of selenium injury was very pronounced in wheat. This difference in the appearance of wheat grown with selenate and with selenite has been pointed out by Hurd-Karrer (*10*). Millet, when grown with selenate applications sufficient to curtail growth markedly, showed at times some yellowing of the leaves, but when grown with similar selenite applications the plants were a normal green.

Other differences in the behavior of sodium selenite and selenate have already been mentioned. All the differences observed are summarized in table 9.

TABLE 9.—*Difference between selenates and selenites*

Respect in which selenate and selenite differ	Selenate	Selenite
How sulfates affect toxicity	Diminish	No effect.
How soil colloids affect toxicity	Practically no effect	Diminish.
Se absorbed, predominately in	Tops	Roots
Symptom produced in wheat	Chlorosis	None[1]

[1] Except where the sulfate supply is insufficient for normal growth

The fact that soil colloids have a marked effect on the toxicity of sodium selenite but practically no effect on the toxicity of sodium selenate may explain observations made in the survey of seleniferous areas. Many plant and soil analyses show that there is little correlation between the total selenium in the soil and the selenium content of the vegetative cover. This might be expected if selenium occurs in soils as both selenate and selenite. The selenate selenium should be highly available in all soils; it should be practically unaffected by soil colloids; and its absorption should vary somewhat with the sulfate content. On the other hand, selenium present as selenite should be only slightly available; its availability should be unaffected by sulfate, but the availability should vary with the kind and quantity of colloidal material present.

SUMMARY

This investigation deals with the effects of soil colloids on the toxicity of sodium selenite to millet as determined by vegetative experiments in quartz sand and sand-soil mixtures. Thirty-two soil samples representative of 22 soil types were tested.

The toxicity of sodium selenite, unlike that of sodium selenate, is not affected by the sulfate supply. It is also independent of the phosphate supply as long as this is sufficient for maximum growth.

The specific effects of different soil colloids on selenite toxicity vary more than tenfold. Their effects bear little relation to the great soil groups. Colloids in two different layers of the same soil have almost identical effects on selenite in some cases, and in other cases the surface-layer colloid has a greater effect than the colloid in the lower layer.

Experiments with several peats failed to show an influence of organic matter on the capacity of the colloid for reducing selenite toxicity, even under conditions of a supersaturated water content. Artificial iron gels had a high capacity for reducing selenite toxicity. A moist gel had a much greater capacity than a dried gel.

The total iron content of soil colloids is correlated with effects of the colloids on selenite toxicity to the degree shown by the coefficient ±0.51. It is suggested that lack of a closer correlation is due to varying reactivities of the iron content.

Plants supplied with sodium selenite contain a higher percentage of selenium in the roots than in the tops. The reverse condition obtains for plants supplied with sodium selenate. Plants grown in quartz sand contain a higher percentage of selenium at half injury than plants grown in sand-soil mixtures.

Rice and millet require about the same concentrations of serenite for half injury; wheat requires about twice as much in both quartz sand and sand-soil mixtures. The fact that the same difference between wheat and millet holds for sand-soil mixtures as for quartz sand suggests that the combination of soil colloid with selenite is slightly available rather than wholly unavailable.

Differences between the effects of selenates and selenites are pointed out.

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PHYSICAL AND CHEMICAL PROPERTIES OF FLANNELS CONTAINING DIFFERENT PROPORTIONS OF NEW AND REPROCESSED WOOL[1]

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INTRODUCTION

Recent efforts to secure "truth in fabrics" legislation, requiring manufacturers to label textiles containing wool with the percentages of new wool present,[3] have led to interest in the quality of fabrics containing new and reprocessed wool in various proportions. South Dakota as a wool-producing State has an interest in variations or differences in the physical and chemical properties of fabrics containing mixtures of new and reprocessed wool. Accordingly in 1938 a study of certain physical and chemical properties of flannel fabrics manufactured from mixtures of new wool and shoddy was initiated. While performance tests of the fabrics have not as yet been conducted, it has been possible to determine the effects of dry cleaning and pressing upon the fabric properties.

Relatively few reports have appeared of investigational work on wool fabrics of known composition. The spinning and weaving qualities of wool from British pedigreed sheep were investigated at the University of Leeds (*7, 8, 9*)[4] and the fiber, yarn, and fabric characteristics determined. Another study of the same type on wool from British breeds of sheep was reported by Barker (*3*) to the New Zealand Government. The Bureau of Animal Industry of the United States Department of Agriculture, in cooperation with the Bureau of Home Economics, has recently published the results of a study of the serviceability of blankets made from four blends of wool (*5*). Wools selected from purebred flocks of Rambouillet and Corriedale sheep and reworked fiber were used in the manufacture of the blankets. The fabrics were given actual service tests, and the rates of chemical and physical deterioration were determined. These Bureaus have in progress further work with blankets involving the use of various grades of reprocessed wool and mohair in comparison with new wool[5] and of suitings made from blends of new wool with reprocessed wool and spun rayon.[6]

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[3] The Wool Products Labeling Act, passed by the 76th Cong., 3d sess., was approved October 14, 1940, and became effective July 14, 1941

[4] Italic numbers in parentheses refer to Literature Cited, p. 597.

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[6] See p. 119 of the reference cited in footnote 4.

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MATERIALS

Rambouillet sheep, raised at South Dakota State College, furnished the new wool used in the present experiment. Ten fleeces weighing approximately 10 pounds each were shipped to the Lowell Textile Institute for fabrication. Reprocessed wool of a high quality from pastel sweater clippings was purchased on the market by members of the institute staff and blended with the new wool. The fiber used for the first fabric (No. 100) was made of 100 percent new wool; for the second (No. 75), approximately 75 percent new wool and 25 percent reprocessed wool; for the third (No. 50), approximately 50 percent new wool and 50 percent reprocessed wool; for the fourth (No. 25), approximately 25 percent new wool and 75 percent reprocessed wool. The exact weights of the fibers are recorded in table 1, which shows that considerable machine waste resulted in the course of the manufacturing. Thus, while the fiber blends indicated above were used as the raw materials for each of the fabrics, these percentages may not have been maintained in manufacture. Further evidences of shifts in these percentages will be indicated in the discussion below.

Emulsion consisting of 75 percent water and 25 percent oil (fat and mineral oil) was applied to the stock in picking. All of the lots were spun to 3½ run Z twist and an identical lay-out was used consisting of No. 9 reed, 4 yarns per dent, 1,560 warps, 43½ inches in reed, and 36 fillings, resulting in a 2 × 2 even-twill fabric. After weaving, each fabric was given a simple finishing process consisting of washing and pressing. The finished yardage and weight as reported by Lowell Textile Institute are shown in table 1.

TABLE 1.—*Weights of new and reprocessed wool fiber used in manufacturing the four experimental fabrics, and length, width, and finished weight of the four fabrics*

Fabric No	Fiber weights used[1]		Finished fabric			
	New wool	Reprocessed wool	Length	Width	Weight	
	Pounds	*Pounds*	*Yards*	*Inches*	*Pounds*	*Ounces*
100	14.4	0.0	20.6	35.5	9	10
75	10.8	3.5	20.4	36.4	9	6
50	7.2	7.0	20.9	36.0	9	4
25	4.0		18.0	36.5	7	8

[1] Total carding and spinning waste for the 4 fabrics was 16 pounds

METHODS

PHYSICAL-PROPERTY DETERMINATIONS

Each of the four fabrics manufactured was tested physically and chemically as it was received from the manufacturer and after it had been commercially dry-cleaned and pressed 15, 30, and 45 times. The process used in dry cleaning was described as consisting of running in dry-cleaning solvent (Stoddard solvent) in a regular cylindrical dry-cleaning washer for 10 minutes with paste soap and rinsing for 20 minutes on a filter so constructed as to remove the heavy "soil" from the solvent. The solvent was passed continuously from the the washer through the filter and returned again to the washer. After each cleaning the fabrics were pressed on a regular steam press.

Throughout this investigation all testing requiring the maintenance of moisture equilibrium in the fabrics was conducted in a conditioning room maintained at a standard atmosphere of 65 percent relative humidity ±2 percent at 70° F. ±2, as specified by the American Society for Testing Materials (*2, p. 184*). All samples were conditioned for a minimum of 6 hours before testing. Samples used in determining weight per square yard and yarn number were dried to constant weight at 105° C. Yarn-count and twist determinations were conducted under prevailing atmospheric conditions, as were all fiber studies.

The means and standard deviations of measurements were calculated in all instances possible, and the analysis of variance for significance of difference between means was applied in some cases.

A 1-pound sample of scoured new-wool fiber was taken from various parts of the lot for use in measurements of fiber diameter, contour, length, and crimp, and a similar sample was taken from the reprocessed wool in the oil. Portions of the fiber samples were hand-carded and carefully blended. By means of intestinal forceps, recommended by Townsend (*11*) for sampling fiber for length determinations, repeated draws were made and blended until samples of the correct size for each type of fiber measurement were secured.

A modification of the cross-section method recommended by the American Society for Testing Materials (*2, p. 174*) was used to determine the diameter and contour of the new and reprocessed wool. The device described by Hardy (*1*) was used in preparing the cross-sectional microscopic slides. The cross-section mounts were placed on a microscope stage set at right angles to the base and, by the use of strong artificial light, their images were projected onto a ground-glass plate placed at such a distance from the microscope that each fiber section was magnified approximately 500 times. One thousand fiber sections were measured to an accuracy of one micron, as recommended by the American Society for Testing Materials (*2*), at (1) their greatest diameters and (2) the diameters at right angles to their greatest diameters.

The samples for determination of fiber length were prepared according to the method described by Townsend (*11*). Three groups of 150 fibers each were measured to the nearest 0.25 inch by extending the fiber sufficiently to remove the crimp but not to stretch it.

The number of crimps in each fiber measured for length was counted over the entire length, and the average number of crimps per inch was calculated.

Yarn number in the present study was recorded as the number of thousands of yards per pound (typp system). Prior to the determination three 10-yard lengths were dried to constant weight. In the preparation of the samples an attempt was made to measure lengths of yarn from which the crimp had been removed without stretching the yarn. The resulting values were calculated in typp to 13 percent regain- the standard regain for woolen yarn (*2*)- and the results used in the adjustment of tension in determining yarn twist.

The average number of twists per inch was determined using the new-type Suter precision twist tester with spinning twist attachment. The gage length was 10 inches and 50 yarns per sample were measured.

A Scott single-strand tester with autographic recorder was used in the determination of yarn stength. The clamps were set 10 inches apart and moved at a speed of 12 inches per minute. Tensile strength and elongation of yarns removed from the fabric at scattered points were measured in each instance.

Wherever the nature of the test made such measurements possible, samples of both warp and filling sections of fabric were prepared. With the exception of the method for abrasion, all methods were standard procedures of the American Society for Testing Materials (*2*).

Two-inch squares taken at five scattered positions on each fabric were composited for the determination of fabric weight. All of the results were calculated to 13-percent regain and expressed in ounces per square yard.

Measurements of thickness were made on each of 10 grab tensile-strength samples by means of the Schiefer compressometer with a ⅝-inch foot at a pressure of 3.4 pounds per square inch. The results were recorded in the number of 0.001 inch of thickness.

A Lowinson counter was used for the determination of the number of yarns per inch. The 10 samples of fabric prepared for the determination of strength by the strip method were measured for a distance of 1 inch.

A 10-inch square was marked on each fabric before it was sent to a commercial dry cleaner and was measured on its return. Thus shrinkage is reported in percentage shrinkage after 15, 30, and 45 dry cleanings and pressings.

J-type Scott testers with recording serigraphs were used throughout for the determination of the tensile strength and elongation of the experimental samples A 150- to 300-pound-capacity machine was used for the grab samples and a 55- to 110-pound-capacity machine for the strip samples. Ten strips of fabric to form the sample were cut 1½ inches wide by 6 inches long and were raveled to 1 inch. Two-inch fabric jaws set 3 inches apart were employed in the strip tests. Ten grab tensile-strength samples per fabric were cut 4 inches wide and 6 inches long. One-inch front and 3-inch back jaws were used for the grab determinations. On both machines the jaws traveled at the rate of 12 inches per minute. Tensile-strength values were recorded in pounds and elongation in inches.

The ball-burst attachment for the Scott tester was used for the determination of bursting strength. Metal balls 1 inch in diameter and 1¾-inch rings were employed. Ten determinations were made on each fabric, and the results were recorded in terms of force in pounds required to thrust the ball through the fabric. Stretch was recorded by means of the serigraph.

The test specimens used in the determination of tearing strength by the tongue method were cut 3 by 8 inches in size. Three-inch longitudinal cuts were made lengthwise of the specimens from the center of one of the edges. The 55-pound-capacity Scott tester was employed, and the autographic recording device was used to record the average load necessary to break. A planimeter was used in calculating the average tearing strength of the fabrics.

Ten samples of each fabric 1⅞ by 10 inches in size were abraded on the Wyzenbeck precision wear testmeter, with a monel-metal screen used as the abrasive surface. The fabrics were given 1,000 double rubs at 4 pounds pressure at an initial tension of 3 pounds. After abrasion, the tensile strength (strip) of the samples was determined by the methods already outlined.

CHEMICAL-PROPERTY DETERMINATIONS

Samples of fiber and fabrics in the various conditions studied were analyzed in duplicate for their moisture, ash, sulfur, and nitrogen content. Both fiber and yarn were cut into smal pieces to facilitate adequate sampling. Moisture was determined by drying to constant weight in a drying oven at 40° C. One-gram samples were ashed to constant weight and total ash recorded in percentage. The Parr Bomb was used in determining the total sulfur of 0.5-gm. samples, and the percentage of sulfur was calculated. The percentage of total nitrogen was determined upon 1-gm. samples by the Kjeldahl method, with copper sulfate used as the catalyst.

PRESENTATION AND DISCUSSION OF EXPERIMENTAL RESULTS

In the discussion of the results of this investigation, consideration is given first to the properties of the new and reworked fibers. Chemical determinations upon the fabrics after progressively greater numbers of dry cleanings were made, and the results of the application of the analysis of variance to the physical measurements of fabric strength are then discussed.

TABLE 2 *Mean and standard-deviation values for length, crimp, diameter, and contour measurements of new and reprocessed wool*

Wool fiber	Length		Crimp per inch		Diameter		Contour ratio	
	Mean[1]	Standard deviation	Mean[1]	Standard deviation	Mean[2]	Standard deviation	Mean[2]	Standard deviation
	Inch	*Inch*	*Number*	*Number*	*Microns*	*Microns*		
New	2.49	0.96	13.34	3.03	21.16	5.03	1.25	0.143
Reprocessed	1.78	.88	11.95	5.00	25.72	7.22	1.25	.193

[1] 450 determinations.
[2] 1,000 determinations.

COMPARISON OF NEW AND REPROCESSED WOOL FIBERS

The physical characteristics of the new and reprocessed wool fibers are shown in table 2. From these data it may be seen that the average length of the new fiber was 40 percent greater than that of the reprocessed wool and the average number of crimps per inch was over 10 percent greater in the new wool. According to the blood system of grading wool, the virgin wool would be classed as fine and the reprocessed wool as half blood. No difference in average contour ratio was found in the wool. In all the physical measurements made, relative variability was greater in the reprocessed than in the new wool

TABLE 3.—*Ash, nitrogen, and sulfur content of the new and reprocessed wool fiber, on a moisture-free basis*

Wool fiber	Ash	Nitrogen	Sulfur
	Percent	*Percent*	*Percent*
New	0.98	13.50	3.52
Reprocessed	.41	12.63	3.71

The ash, nitrogen, and sulfur content was determined for the new- and reprocessed-wool fibers with the results given in table 3.

The ash content of the new wool was more than twice that of the reprocessed wool, and the nitrogen content was approximately 1 percent greater. The sulfur content was 0.19 percent lower in the new wool.

COMPARISON OF FABRICS MANUFACTURED FROM NEW AND REPROCESSED WOOL

PHYSICAL AND CHEMICAL PROPERTIES OF FABRICS AS RECEIVED FROM THE MANUFACTURER

In the discussion of the manufacture of the fabrics it was noted that identical methods were used throughout in the preparation of yarns and in the weaving and finishing processes. When the finished materials were examined, it was evident that the fabrics produced from the various blends did not have the same properties. The data in table 4 shows that the average weight per square yard decreased as the percentage of reprocessed wool increased. Thickness and twist were approximately the same throughout the series, and the number of yarns per inch decreased slightly with increasing percentages of reprocessed wool.

In discussing the relative merits of the four fabrics, the fact that inherent differences were present in them must be kept in mind. Since correction factors accounting for the complexity of factors are not available, the data reported are those of the actual results obtained, the constant factors being identical manufacture and dry-cleaning processes rather than identity in finished fabric.

TABLE 4.—*Mean values for characteristics of the 4 fabrics containing different percentages of new and reprocessed wool fiber*

Fabric No.[1]	Weight per square yard[2]	Thickness	Yarns per inch		Shrinkage[3]		Twists per inch	
			Warp	Filling	Warp	Filling	Warp	Filling
	Ounces	*0.001 inch*	*Number*	*Number*	*Percent*	*Percent*	*Number*	*Number*
100-0	7.92	32.2	44.3	36.2			11.7	12.4
100-15	8.75	35.4	45.2	38.5	5.0	1.2	12.5	12.8
100-30	8.94	36.5	45.9	39.3	6.2	1.2	12.8	12.9
100-45	8.48	36.0	45.3	39.3	6.2	1.2	12.9	12.8
75-0	7.71	32.8	43.3	35.9			12.2	12.4
75-15	8.45	36.0	44.2	37.9	3.8	1.2	12.3	12.4
75-30	8.69	36.4	44.8	38.0	6.2	1.2	12.7	12.4
75-45	8.90	36.2	44.7	38.3	6.2	1.2	12.6	12.5
50-0	7.36	33.0	43.0	35.4			12.4	12.5
50-15	7.77	35.2	43.9	37.2	3.8	1.2	12.6	12.6
50-30	8.29	35.4	44.5	38.5	6.2	1.2	12.5	12.6
50-45	7.76	35.2	43.9	38.2	6.2	1.2	12.5	12.6
25-0	6.71	31.6	42.9	35.3			12.1	12.4
25-15	7.01	33.8	43.5	38.2	3.8	1.2	12.0	12.2
25-30	7.32	33.2	43.7	37.8	5.0	1.2	12.2	12.4
25-45	7.28	33.6	43.4	38.2	5.0	1.2	12.3	12.7

[1] [illegible] the fabrics.

[2] 13 percent regain.

[3] Caused by dry cleaning and pressing

Since fabric weight decreased progressively with increasing percentages of reprocessed wool, it must be concluded that there was more carding and spinning waste as the percentage of reprocessed wool was increased. In this same connection, a comparison of the average diameter of the fiber present in the finished fabrics is of significance. The results of this study are shown in table 5. From these data it is apparent that in the case of the 100-percent virgin fabric the manufacturing processes did not affect either the average diameter or the distribution as measured by the standard deviation. It would be anticipated that the average diameter of the wool present in the fabrics containing reprocessed wool would increase, approaching that of the reprocessed fiber as the percentage of reprocessed fiber became higher. However, as is shown in table 5, the diameter increased but slightly. Thus in the manufacturing processes the coarser reprocessed fiber must have been eliminated to a certain extent. Since the contour ratios of the new and the reprocessed fiber were the same, no change in contour ratio would be anticipated throughout the series of fabrics; and, in fact, the actual differences found are not to be considered significant from a practical standpoint.

Table 5.—*Diameter and contour ratios of the fibers in the new and reprocessed wool and in the manufactured fabrics*

Sample[1]	Diameter		Contour ratio	
	Mean	Standard deviation	Mean	Standard deviation
	Microns	*Microns*		
New fiber	21.16	5.03	1.25	0.143
Reprocessed fiber	25.72	7.22	1.25	.193
Fabric No.—				
100	21.30	5.16	1.24	.140
75	21.71	5.52	1.24	.142
50	21.77	5.73	1.25	.158
25	22.04	6.56	1.23	.139

[1] Size of sample, 1,000.

EFFECTS ON FABRICS OF DRY CLEANING AND PRESSING

In table 6 are shown the results of the ash, nitrogen and sulfur analyses of the four fabrics as received from the manufacturer and after various numbers of dry cleanings and pressings. It will be noted that, with few exceptions, the tendency was for the percentage of ash to increase with dry cleaning. In all four fabrics there was a marked increase in nitrogen between the fifteenth and thirtieth dry cleanings, an increase which could not be accounted for on the basis of any of the other physical or chemical experimental results. It has

Table 6.—*Ash, nitrogen, and sulfur content on a moisture-free basis of the four fabrics containing different percentages of new and reprocessed wool fiber*

Fabric No.	Ash	Nitrogen	Sulfur	Fabric No.	Ash	Nitrogen	Sulfur
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
100-0	0.48	13.55	3.34	50-0	0.64	13.50	3.47
100-15	.97	13.15	3.54	50-15	1.02	13.38	3.39
100-30	.94	15.83	3.37	50-30	.91	15.99	3.36
100-45	1.08	15.78	3.74	50-45	1.14	15.67	3.43
75-0	.66	13.58	3.63	25-0	.61	13.42	3.44
75-15	.83	13.07	3.59	25-15	.96	13.15	3.33
75-30	.91	15.66	3.58	25-30	.84	15.55	3.41
75-45	1.00	15.45	3.34	25-45	1.36	15.57	3.27

been suggested that accumulation of the paste soap might have been responsible, but no analysis of the soap is available Sulfur determinations indicate that no fabric blend or treatment affected the results to a greater or less extent than any other throughout the series In some instances a fabric sample had a higher sulfur content as a result of one stage of treatment, whereas in other fabrics this treatment apparently caused a lowering of the sulfur content.

In the discussion of the effects of abrasion and dry cleaning and pressing, statistical methods have been applied in the comparisons of the four fabrics in the various stages of treatment All of the data reported in tables 7 to 10 are involved in the calculations.

TABLE 7 —*Mean and standard-deviation values for the yarn strength and elongation measurements of the experimental fabrics as affected by dry cleaning and pressing*

Fabric No[1]	Yarn strength				Yarn elongation			
	Warp		Filling		Warp		Filling	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
	Grams	*Grams*	*Grams*	*Grams*	*Centimeters*	*Centimeters*	*Centimeters*	*Centimeters*
100 0	311 2	72 02	287 6	75 90	5 09	0 929	5 06	0 890
100 15	337 9	49 52	285 7	49 49	4 88	842	4 61	874
100-30	319 5	52 45	327 9	47 61	4 67	1 023	4 86	960
100-45	317 2	42 63	300 2	42 12	4 32	857	4 71	830
75 0	268 3	35 55	282 7	27 26	4 38	883	4 76	782
75-15	288 6	46 33	267 5	32 97	4 16	876	4 39	821
75 30	262 8	44 43	267 3	43 12	3 91	869	4 01	919
75 45	261 2	34 08	248 9	30 05	4 05	634	4 18	693
50 0	224 0	36 10	196 4	24 54	3 92	781	3 83	879
50 15	222 6	29 60	193 0	20 15	3 52	665	3 68	679
50 30	221 6	36 18	196 8	32 71	3 47	790	3 69	712
50-45	213 7	32 67	196 4	28 57	3 73	669	3 25	605
25-0	171 5	31 68	181 0	35 34	2 89	693	3 27	723
25-15	169 5	32 78	164 1	29 21	2 66	661	2 91	676
25-30	179 8	26 99	148 3	28 41	2 64	625	2 65	609
25-45	170 3	30 99	149 7	24 79	2 78	676	2 79	693

[1] Size of sample 50

TABLE 8 *Mean and standard-deviation values for strip tensile-strength and elongation measurements of the experimental fabrics before and after abrasion*

BEFORE ABRASION

Fabric No[1]	Strip breaking strength				Strip elongation			
	Warp		Filling		Warp		Filling	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Inches*	*Inches*	*Inches*	*Inches*
100-0	36 4	1 18	27 2	0 86	0 84	0 149	1 07	0 066
100-15	37 0	1 01	30 6	1 27	1 05	202	1 08	094
100 30	36 8	68	30 9	1 39	98	081	1 10	081
100-45	35 7	95	28 4	1 31	97	066	1 06	094
75 0	30 2	63	24 1	92	71	056	99	077
75-15	31 2	1 14	26 8	1 11	85	058	1 11	128
75 30	30 4	1 17	25 7	1 93	80	066	93	316
75-45	31 2	1 18	23 6	1 02	85	100	93	310
50 0	25 7	89	19 0	67	58	094	83	081
50-15	27 0	67	19 8	53	65	058	84	047
50-30	24 1	1 22	21 4	64	70	066	88	047
50-45	27 4	88	20 0	60	66	066	81	034
25 0	21 2	47	15 1	78	44	047	63	047
25-15	22 2	75	17 5	1 29	60	000	72	081
25-30	21 6	1 14	16 0	83	62	047	72	047
25 45	21 0	68	16 0	53	02	060	73	047

[1] Size of sample 10

TABLE 8.—*Mean and standard-deviation values for strip tensile-strength and elongation measurements of the experimental fabrics before and after abrasion*—Con.

AFTER ABRASION

Fabric No.[1]	Strip breaking strength				Strip elongation			
	Warp		Filling		Warp		Filling	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Inches*	*Inches*	*Inches*	*Inches*
100-0	35.6	0.63	28.7	1.36	0.79	0.088	1.01	0.111
100-15	37.0	1.01	31.2	1.51	.87	.066	.99	.058
100-30	37.0	.68	30.4	1.11	.98	.081	1.04	.066
100-45	35.4	.58	30.4	1.85	.91	.100	1.02	.081
75-0	40.2	1.09	25.3	.89	.65	.089	.84	.081
75-15	31.0	.55	26.6	1.46	.73	.066	.91	.075
75-30	30.6	.83	26.4	.98	.80	.081	.95	.058
75-45	31.1	1.93	26.8	1.51	.78	.066	.89	.075
50-0	26.8	.43	20.2	.79	.53	.047	.74	.047
50-15	26.8	.72	21.0	.91	.64	.047	.75	.075
50-30	26.5	.74	21.0	.53	.66	.047	.73	.047
50-45	25.8	1.00	21.8	.97	.69	.058	.72	.045
25-0	21.8	.75	16.0	1.05	.45	.058	.61	.058
25-15	21.6	.52	16.4	.81	.52	.047	.63	.047
25-30	21.3	.42	16.0	1.03	.54	.047	.60	.047
25-45	20.8	.89	16.6	.70	.55	.058	.61	.058

[1] Size of sample, 10.

TABLE 9.—*Mean and standard-deviation values for grab breaking-strength and elongation measurements of the experimental fabrics as affected by dry cleaning and pressing*

Fabric No.[1]	Grab breaking strength				Grab elongation			
	Warp		Filling		Warp		Filling	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Inches*	*Inches*	*Inches*	*Inches*
100-0	52.0	1.25	43.8	2.30	0.79	0.058	1.03	0.066
100-15	52.1	1.81	45.8	2.19	.82	.047	1.06	.066
100-30	53.7	2.10	47.8	2.83	.90	.081	1.16	.133
100-45	49.6	1.63	44.0	2.81	.90	.081	1.05	.100
75-0	42.5	2.51	35.4	2.49	.77	.094	.99	.075
75-15	42.9	1.37	40.4	1.45	.80	.081	.97	.061
75-30	42.5	1.58	37.0	1.82	.83	.064	.92	.047
75-45	42.0	1.05	37.0	1.95	.76	.066	.93	.066
50-0	35.6	2.77	29.0	2.03	.66	.061	.95	.145
50-15	35.9	1.85	29.1	1.43	.65	.058	.85	.088
50-30	35.5	1.81	29.5	1.65	.70	.047	.83	.066
50-45	34.5	1.03	28.8	1.77	.68	.094	.80	.094
25-0	27.0	1.04	22.6	1.10	.45	.058	.73	.066
25-15	27.8	.88	24.2	1.12	.55	.058	.73	.064
25-30	28.1	1.10	23.0	1.09	.54	.047	.69	.075
25-45	26.6	1.22	23.1	.97	.53	.047	.67	.047

[1] Size of sample, 10.

TABLE 10.—*Mean and standard-deviation values for the bursting-strength, bursting-elongation, and tearing-strength measurements of the experimental fabrics as affected by dry cleaning and pressing*

Fabric No [1]	Bursting strength		Bursting elongation		Tearing strength			
					Warp		Filling	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
	Pounds	*Pounds*	*Inches*	*Inches*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
100-0	81 4	2 92	0 50	0 000	5 85	0 242	6 10	0 400
100-15	88 6	4 67	52	079	4 80	350	5 05	370
100-30	88 6	2 57	.50	000	5 15	338	4 85	338
100-45	87 7	2 89	50	000	4 90	316	4 50	236
75-0	77 6	3 14	52	079	4 95	160	4 80	350
75-15	78 8	2 81	48	079	4 35	242	4 20	258
75-30	76 0	2 83	42	120	4 35	242	4 40	.316
75-45	78 9	2 45	48	079	4 30	258	4 35	242
50-0	65 1	2 21	48	079	4 50	236	4 40	211
50-15	65 6	1 61	40	128	4 05	160	3 75	264
50-30	66 9	3 10	45	105	4 05	100	3 65	242
50-45	67 0	2 71	48	079	4 05	285	3 85	242
25-0	56 2	3 84	48	079	3 95	160	4 20	258
25-15	60 6	1 44	48	079	3 85	242	3 65	242
25-30	60 3	2 44	50	000	3 90	316	3 80	258
25-45	59 0	2.27	40	128	3 95	285	3 75	264

[1] Size of sample, 10

After subjecting samples of each of the experimental fabrics to mechanical abrasion, strip tensile-strength samples were cut and tensile strength following abrasion for 1,000 double rubs was determined. The results of these measurements are recorded in table 8. "Student's" *t* test for significance of difference of means has been applied in comparing the strength and elongation values for the fabrics before and after the abrasion treatment (table 8). Nonsignificant differences were found in both warp and filling determinations of strength. In elongation measurements the means of both warp and filling determinations were found to differ significantly. Thus, with five exceptions, elongation was found to decrease after abrasion without causing a decrease in strength.

Since homogeneity of errors is assumed in the calculations of the analysis of variance, the χ^2 test was used to determine whether the variability of the strength and elongation measurements for the fabrics subjected to the various numbers of dry cleanings and pressings were homogeneous (Snedecor, *10*). The results indicated in table 11 show that this was true in all instances.

TABLE 11.—*Analysis of errors of strength and elongation measurements of the fabrics by means of the* χ^2 *test for homogeneity*

Measurement	χ^2 for			
	Strength		Elongation	
	Warp	Filling	Warp	Filling
Yarn	3 99	4 45	2 86	2 62
Strip				
Before abrasion	2 74	3 67	3 44	2 04
After abrasion	2 63	3 70	3 34	2 61
Grab	2.82	4 60	2 53	1 75
Bursting [1]	2 21		50	-
Tear	1 08	1 54		

[1] Fabric direction not involved in bursting-strength determination.

The analysis of variance suggested by Fisher (4) was applied to the measurements of yarn and fabric strength and elongation in order to test for significance of differences between fabrics and treatments (dry cleaning and pressing) There were 4 fabrics receiving 4 treatments, 10 measurements being made for fabrics and 50 for yarns. The degrees of freedom are shown in table 12

TABLE 12 *Degrees of freedom involved in the analysis of variance of the measurements of yarn and fabric strength and elongation*

Variation due to	Degrees of freedom	
	Fabric studies	Yarn studies
Blend	3	3
Treatment	3	3
Blend × treatment	9	9
Residual error	144	784
Total	159	799

In the analysis of the data based upon these tests of significance (table 13) consideration is given first to the cases in which the residual component is used as error and finally to those in which blend × treatment is used as error If the residual is used as error, the interpretation applies to the specific fabrics and treatments involved in this study However it is of interest to expand the interpretation to

TABLE 13 *F values by means of the analysis of variance for yarn- and fabric-strength and for yarn- and fabric-elongation determinations, using residual and interaction as errors*

STRENGTH DETERMINATIONS

Measurement and source of variation	Degrees of freedom	F values			
		Warp		Filling	
		Residual as error	Interaction as error	Residual as error	Interaction as error
Yarn strength					
Blend	3	[1] 565.84	[1] 310.82	[1] 675.32	[1] 64.55
Treatment	3	[1] 5.66	3.11	[1] 5.2	.52
Blend × treatment	9	1.82		[1] 10.15	
Strip strength before abrasion					
Blend	3	[1] 1,857.98	[1] 1,074.97	[1] 1,166.64	[1] 184.96
Treatment	3	[1] 10.90	[1] 6.30	[1] 45.25	[1] 7.17
Blend × treatment	9	1.73		[1] 6.31	
Strip strength after abrasion					
Blend	3	[1] 2,076.27	589.05	[1] 1,134.07	[1] 773.76
Treatment	3	[1] 6.15	1.74	[1] 11.44	[1] 7.80
Blend × treatment	9	[1] 3.52		1.48	
Grab strength					
Blend	3	[1] 1,573.91	[1] 869.96	[1] 1,069.77	[1] 476.14
Treatment	3	[1] 8.84	[2] 4.89	[1] 12.32	[2] 5.48
Blend × treatment	9	1.81		[2] 2.25	
Tearing strength					
Blend	3	[1] 181.91	[1] 32.09	[1] 165.05	[1] 23.03
Treatment	3	[1] 39.66	[1] 7.00	[1] 62.37	[1] 8.70
Blend × treatment	9	[1] 5.67		[1] 7.17	
Bursting strength [3]					
Blend	3	[1] 681.36	[1] 201.47		
Treatment	3	11.25	3.29		
Blend × treatment	9	[1] 3.36			--

See footnotes at end of table

TABLE 13.—*F values by means of the analysis of variance for yarn- and fabric-strength, and for yarn- and fabric-elongation determinations, using residual and interaction as errors*—Continued

ELONGATION DETERMINATIONS

Measurement and source of variation	Degrees of freedom	*F* values: Warp: Residual as error	*F* values: Warp: Interaction as error	*F* values: Filling: Residual as error	*F* values: Filling: Interaction as error
Yarn elongation					
Blend	3	[1]230.91	[1]137.67	[1]218.44	[1]102.50
Treatment	3	[1]11.45	[2]6.83	[1]18.15	[1]8.52
Blend × treatment	9	1.68		[2]2.13	
Strip elongation before abrasion					
Blend	3	[1]155.25	[1]56.54	[1]204.26	[1]54.50
Treatment	3	[1]20.30	[1]7.44	[1]8.35	2.23
Blend × treatment	9	[1]2.74		[1]3.75	
Strip elongation after abrasion					
Blend	3	[1]223.95	[1]229.54	[1]286.07	[1]160.64
Treatment	3	[2]3.55	3.64	1.53	.86
Blend × treatment	9	.98		1.78	
Grab elongation					
Blend	3	[1]186.48	[1]93.14	[1]137.27	[1]42.62
Treatment	3	[1]8.43	[2]4.21	3.80	1.18
Blend × treatment	9	[2]2.00		[1]3.22	
Bursting elongation [3]					
Blend	3	[2]3.41	1.67		
Treatment	3	1.13	.55		
Blend × treatment	9	2.03			

[1] Exceeds 1-percent point

[2] Exceeds 5-percent point

[3] Fabric direction not involved in bursting-strength determinations

indicate the general effects of mixing new and reprocessed wool and the effect of dry-cleaning and pressing processes. As has been suggested by Immer (*6*), the interaction is the proper error in this case. When the residual is used as error, the blends are found to differ significantly in strength and elongation in all of the yarn and fabric measurements (table 13). Highly significant differences would be anticipated by examination of tables 7 through 10, reporting the actual strength and elongation results. It may further be noted from these data that, in general, strength decreased regularly as the percentage of reprocessed wool became greater.

With the exception of bursting elongation, in which significant differences were found between the blends, highly significant differences between blends were present. Thus, as is shown in table 7 through 10, elongation decreased with increasing percentages of reprocessed fiber.

If the residual component is used as error in testing for the effects of dry cleaning and pressing (treatment), highly significant differences are found in all instances of strength determinations and in two instances of elongation determinations. This may be seen by examination of the data in table 13.

If consideration is given to the totals for blends receiving the same treatment, the reason for the existence of significant differences between treatments becomes evident. The strength measurements are found to be greater at 15 dry cleanings, with progressively decreasing values after 30 and 45 dry cleanings in five cases; values decrease throughout in the case of the tearing-strength measurements in the filling direction; increase at 15 dry cleanings, decrease at 30, and again increase at 45, following abrasion, in the filling direction; increase progressively through 30 dry cleanings and decrease at 45 dry cleanings in grab and abrasion measurements of the warp; while two measurements, tearing strength of the warp and filling, show marked decreases after 15 dry cleanings, remain approximately the same through 30 dry cleanings, and then decrease after 45 dry cleanings.

The effects of dry cleaning and pressing on elongation are not necessarily of the same order as those on the strength measurements. The totals are found to increase after 15 dry cleanings and then gradually to decrease in the filling direction of the grab determinations; to increase at 15 dry cleanings and remain approximately the same throughout in the warp direction of the strip determinations; to increase through 30 dry cleanings and decrease at 45 dry cleanings in the warp and filling abrasion and warp grab measurements; and to decrease progressively in the remaining four instances.

The third question of interest is whether the effect of treatment upon the four blends was similar. The test of significance is made by dividing the mean square for interaction by the error mean square. These results are also recorded in table 13. From this table it may be seen that in the strength measurements highly significant interactions are found in six instances, significant interactions in one instance, and nonsignificant interations in four instances. Treatment does not affect elongation significantly in three cases, while significant differences are found in three instances, and highly significant differences in three others.

A detailed study of the means indicates the reason for significant differences in the results. For example, the mean of the warp abrasion-strength measurements for fabric 100 is greater at 15 dry cleanings, the same at 30 as at 15 dry cleanings, and lower at 45 than at 30 dry cleanings; for fabric 75 it is lower at 15, lower still at 30, and at 45 approximately the same as at 15; for fabric 50 it is the same at 15 as before dry cleaning, slightly lower at 30, and lower still at 45; and for fabric 25 it is slightly lower after each series of dry cleanings. Similar fabric behavior is found in the elongation measurements, where significant or highly significant results are recorded. While each individual fabric does not vary a great deal in its reaction to treatment, the dissimilar reaction of the other fabrics results in the significant differences found between treatments.

Thus, analysis of the data with the residual used as error leads to the conclusions that the four fabrics were significantly different in measurements of strength and elongation; the treatments to which the fabrics were subjected were significantly different; and in some instances the four fabrics did not react similarly to treatment as registered in terms of strength and elongation. Tables of the actual results show that the fabrics decreased in strength in progressing from the 100 percent new-wool fabric to the 25 percent new-wool fabric when similar manufacturing processes were employed. Elongation likewise decreased as the percentage of reprocessed fiber was increased. Dry cleaning and pressing increased strength in some instances and lowered it in others.

As is suggested above, it is desirable to expand the interpretation of the data to include an analysis of the effects of dry cleaning and pressing on flannel fabrics containing different percentages of new and reprocessed wool. For this purpose the interaction of blend × treatment is used as error. The results of these calculations are also reported in table 13. In making these interpretations, however, it is recognized that the inclusion of a larger number of blends and treatments would have been desirable and that the results are applicable only to blends in which the fiber properties are those found in this study. Thus all blends of new and reprocessed wool fiber would not necessarily produce fabrics having the properties found in this study.

Upon examination of the *F* values it may be seen that, regardless of treatment, varying the blend of new and reprocessed fibers appreciably in flannel fabrics yielded fabrics that differed significantly in strength and elongation. One exception to this finding was the elongation measurements during the determination of bursting strength, where it was found that all blends behaved similarly.

A second determination of importance involved testing for the significance of fabric treatment. In the strength measurements it is found that, regardless of blend, treatment effect is highly significant in four cases, significant in four, and nonsignificant in three With the exception of tearing strength, in which both warp and filling directions are highly significant, the filling direction of the fabrics shows a greater tendency than the warp to give highly significant results. In several instances fabrics that are significantly different in one fabric direction are nonsignificant in the other. Thus the results of the application of the test of significance for the effects of fabric treatment on the strength of the fabrics are mixed.

In the effect of treatment on fabric elongation it is found that, regardless of blend, treatment affects elongation highly significantly in two instances, significantly in two, and nonsignificantly in five.

In summarizing the findings based on the second method of interpretation, it is shown that blending new and reprocessed wool fiber of the types employed in the present study in varying proportions and subjecting them to the same manufacturing processes resulted in fabrics of different strength and elongation, regardless of subsequent dry-cleaning and pressing processes. Dry cleaning and pressing the fabrics affected strength and elongation significantly in some instances and nonsignificantly in others, regardless of the blend.

SUMMARY

Wool flannel fabrics containing different blends of new and reprocessed fiber were manufactured, using identical spinning, weaving, and finishing processes. Chemical and physical tests were applied to the fabrics as received from the manufacturer and after varying numbers of commercial dry-cleanings and pressings.

Comparisons of the fiber qualities of the new and the reprocessed wool indicated that the new wool was finer, longer, and crimpier. No difference in average contour ratio was found.

Ash and nitrogen, were greater in the new fiber whereas the sulfur content was lower.

Examination of the fundamental properties of the four fabrics manufactured indicated that, when fibers of the nature described were used for blending, an increase in the percentage of reprocessed wool resulted in an appreciable decrease in the weight per square yard, though the number of yarns per inch decreased only slightly and the twist remained approximately the same.

Comparisons between the diameter of the fibers in the finished fabrics and those in the new and reprocessed wool from which the fabrics were made indicated that the coarser reprocessed fiber must have been eliminated to a certain extent during manufacture.

Determinations of the moisture, ash, nitrogen, and sulfur content of the fabrics before and after dry cleaning and pressing showed that the ash tended to increase with dry cleaning; that neither blend nor treatment affected the sulfur content appreciably; and that in all blends there was a marked increase in nitrogen between the fifteenth and thirtieth dry cleanings.

Statistical methods were applied in the analyses of the effects of abrasion and dry cleaning and pressing on the four fabrics. Nonsignificant differences in strength between unabraded fabrics and fabrics abraded 1,000 times were found. However, there was a decrease in elongation following abrasion.

When the interpretation of the results of the application of the analysis of variance was limited to comparisons between the four fabrics studied, it was found that the fabrics were significantly different in strength and elongation; the treatments to which they were subjected were significantly different; and in some instances they did not react similarly to treatment. Expanding the interpretation to indicate the general effects of blending new and reprocessed wool of the types used in this study in manufacturing flannel fabrics resulted in the conclusion that fabrics of different strength and elongation are produced, regardless of subsequent dry cleaning and pressing processes to which they may be exposed. Thus, regardless of the method of interpretation, it was found that increases in the percentage of the reprocessed wool used in this study resulted in corresponding decreases in fabric strength and elongation. The effects of dry cleaning and pressing were mixed and therefore inconclusive.

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INHERITANCE OF RESISTANCE TO SIX PHYSIOLOGIC RACES OF BEAN RUST[1]

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INTRODUCTION

The presence of 2 physiologic races of the bean rust organism (*Uromyces phaseoli typica* Arth.) was first demonstrated by Harter, Andrus, and Zaumeyer (*6*).[3] Later, Harter (*5*) and Harter and Zaumeyer (*7*) reported 18 additional ones. They showed that certain varieties and strains of beans (*Phaseolus vulgaris* L.) were resistant to a number of these physiologic races whereas other varieties were susceptible.

Wingard (*13*) studied the inheritance of rust resistance and showed in two different crosses involving resistance and susceptibility that resistance was dependent on a single dominant factor, the F_2 results showing a ratio of 3 resistant plants to 1 susceptible. He made no counts in the F_3 generation, but reported that the susceptible F_2 plants bred true for susceptibility in the F_3 generation and that the F_2 resistant plants segregated for resistance and susceptibility. It is assumed that Wingard worked with a single physiologic race, and his results are accepted as proof of the mode of inheritance for the bean varieties and the race of rust he used. With the identification of a number of physiologic races of bean rust since the publication of his results, the question naturally arose as to whether progenies of various bean crosses show the same genetic behavior with reference to other races of rust. Investigations (*1*, *2*, *4*, *11*) on the stem rust of wheat (*Puccinia graminis tritici* Eriks. and Henn.) showed that resistance was not inherited alike by all varieties and to various races of the parasite. This paper deals with the inheritance of resistance, in a number of bean hybrids, to several of the physiologic races of the bean rust organism.

MATERIALS AND METHODS

The rust material used in these studies was either collected by the writers in several sections of the United States or sent to them by various pathologists throughout the country. Later a number of different bean varieties were inoculated with these strains for purposes of identification and to test the purity of the particular physiologic race. If more than one race were present, each was isolated and pure-lined by using spores of a single sorus. Each race was then increased by the inoculation of some susceptible variety and then reinoculated to the differential varieties for the redetermination of purity.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 621

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When this was accomplished, the infected leaves were collected, dried for a few days between blotting papers, and then stored in envelopes in a refrigerator at −6° C. until needed for inoculation purposes. The physiologic races involved in this study were 1, 2, 6, 11, 12, and 17, and the identical lines of these races were maintained throughout the course of the experiments. In some cases slight race mixtures occurred in the inoculated F_3 plants, but these were in such reduced numbers that they were readily detectable and did not in any way confuse the results. For example, certain plants from a homozygous susceptible line exhibited a very small number of lesions of the resistant type, whereas the great majority of lesions were of the susceptible type, indicating that the resistant lesions were possibly produced by a race of the rust unlike the one producing the large pustules. Similarly, in certain homozygous resistant and heterozygous lines the predominating lesions were of the resistant type, but occasionally a small percentage of the pustules of the extremely susceptible type were noted; this indicated a slight race mixture.

The bean varieties or strains (table 1) used for differentiating the several physiologic races were as follows: (1) U. S. No. 3, a white-seeded Kentucky Wonder type; (2) Small White (*California*); (3) Pinto; (4) No. 765,[4] a Kentucky Wonder Wax selection; (5) No. 780,[4] a white-seeded Kentucky Wonder strain; (6) No 814,[4] a brown-seeded Kentucky Wonder type. These varieties and those used for hybridization were inoculated in progeny tests and were found to breed true for resistance and susceptibility. The seed was grown by the writers at Greeley, Colo. In order to study the mode of inheritance of resistance to several of the physiologic races of bean rust, four different crosses involving six bean varieties and strains were made between certain varieties that were resistant to some of the races and those that were susceptible, and later the progenies were tested with the six selected physiologic races of the organism. One of the determining factors underlying the choice of the physiologic races for study was the reaction of the differential varieties whose resistance and susceptibility to these races had previously been determined. By a consideration of these factors it was believed that a fairly reliable cross section of the reaction to the six races chosen would be obtained, or at least enough would be learned about them to determine whether the hybrids that were inoculated agreed or differed in their mode of inheritance.

TABLE 1.—*Reaction of differential bean varieties and strains to 6 selected physiologic races of bean rust*

Differential variety or strain	Infection grade[1] produced by physiologic race—					
	1	2	6	11	12	17
U. S. No 3	2	10	[2] $\frac{5\ 6}{3}$	10	10	10
Small White (*California*)	9	8	9	9	10	1
Pinto	10	10	10	10	10	10
No 765	1	1	2	6	2	5
No. 780	1-2	1	2	6	3	1
No. 814	1	0	9	10	0	0

[1] Infection grades range from 0 for immunity to 10 for highest degree of susceptibility

[2] The fractional expression of grade indicates unequal size of pustules on upper and lower sides of leaf

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[4] Numbers carried in the files of the writers

Hybridization was done under controlled greenhouse conditions. The parents and the crosses are listed in tables 2 and 3. Instead of inoculating the F_1 plants, the seed was increased under field conditions at Greeley, Colo. The environmental conditions of low humidities and high temperatures that prevail in Colorado and the danger of contamination of the physiologic races made it inadvisable to test the plants in the F_1 generation. The F_2 plants were inoculated under greenhouse conditions at Beltsville, Md. The seed was planted in 4-inch pots, and when the primary leaves were about three-fourths grown they were inoculated with the particular race of rust to be investigated by spraying their upper sides with a spore suspension. At the same time a set of differential varieties was also inoculated to be certain of the purity of the particular race. The inoculated plants were then placed in a large chamber where the humidity was maintained at about 95 percent. After 24 to 48 hours in this chamber, they were removed and placed in greenhouse benches at a temperature varying from 75° to 80° F. At the end of 14 days, the grades of infection were recorded and the plants were labeled as to their reaction to the physiologic race of rust involved. Some of the hybrid plants of all classes were transplanted to greenhouse benches and allowed to grow to maturity. The seed harvested from the transplanted F_2 plants was grown later, and the plants were tested under greenhouse conditions for the F_3 data. The F_2 plants selected to give rise to the F_3 families were chosen at random within the resistant and susceptible classes. The F_3 families resulting from these selected F_2 plants were inoculated, and the results obtained were used to verify the F_2 data.

TABLE 2.—*Reaction of parental bean varieties and strains to 6 selected physiologic races of bean rust*

Variety or strain	Infection grade produced by physiologic race—					
	1	2	6	11	12	17
No. 765	1	1				
Pencil Pod Black Wax	10	10				
No. 780		1		6		
Pinto		10	10	10	10	10
Cranberry			0			
No. 192 48[1]					0	0

[1] A strain closely related to No. 814, one of the differential varieties.

All of the F_2 plants that were inoculated with the six rust races were tested in the spring of 1938 and 1939. Most of the F_3 plants of the various hybrids were tested in the fall of 1939 and the first 3 months of 1940, when conditions were not as favorable for the development of the host and fungus as they were when the F_2 plants were inoculated.

The method used in identifying the various physiologic races was described by Harter and Zaumeyer (*7*) and will be reviewed only briefly. The scale (table 1) for relative resistance or susceptibility ranges from 0 to 10 and is based on the size of the sori. Under very favorable conditions for growth, readings lower than 5 (see figs. 1, *A*; 5, *A*; 7, *B*) denote grades of resistance; those from 5 to 10 (see figs. 1, *B*; 6, *A*) represent grades of susceptibility. There appears to be no trace of mycelial invasion in the host tissue of plants rated 0 (see fig. 3, *A*), and they are consequently regarded as immune. A grade of 1 (see fig. 1, *A* and *C*) denotes a very high degree of resistance with the production of small to medium hypersensitive necrotic areas on the leaves without the formation of uredospores. A plant in grade 2 is still considered highly resistant, but a small number of uredospores are produced in the hypersensitive necrotic lesions. Plants with ratings of grade 3–4 (see figs. 5, *E*; 6, *D*) are moderately resistant, and the sori on the leaves are comparatively small. Plants rated grade 5 or above are regarded as being in the susceptible class, the pustules becoming progressively larger until the extremely susceptible grade 10 (see fig. 2, *B*) is reached. Table 1 shows that each physiologic race can be readily identified from its degree of infectivity on the several differential bean varieties.

The ratings in all classes were found to vary somewhat with changes in environmental conditions. Under favorable conditions, such as bright sunlight and a moderately high temperature (20° to 30° C.) following infection, the various physiologic races produced their maximum development, but during dark, cool weather or at temperatures above 32° the lesions were usually subnormal in size. For example, a variety rating grade 1 or 2 under favorable environment for the fungus may show a 0 or 1 rating, respectively, under adverse conditions. This would also be true for other grades, as, for example, a variety showing a 9 to 10 rating under good conditions might show only 7 to 8 under conditions adverse for the rust or host.

After establishing the purity of the several physiologic races, the parents were inoculated to determine their reaction to the six selected races of rust. These data are recorded in table 2.

The data recorded in this paper were subjected to the χ^2 test for goodness of fit. This test was applied independently to F_2 families from individual F_1 plants and to F_3 families from F_2 plants and also to the totals of a number of tested families according to Fisher's (*8*) statistical methods.

EXPERIMENTAL RESULTS

REACTION OF F_2 GENERATION

The F_1 plants were not inoculated but were grown under field conditions in order to obtain large seed populations. From 160 to 593 plants in the several F_2 progenies were inoculated (tables 3 and 4). No test was made with reciprocal crosses. An analysis of the data (table 3) shows that with races 1, 2, 6, and 12, resistance was dominant to susceptibility, with a 3:1 segregation in the F_2 generation, indicating a single-factor difference. With races 11 and 17, incomplete dominance is shown by the segregation of resistant, intermediate, and susceptible plants in the ratio of 1:2:1 (table 4). The χ^2 values indicated that the observed data fit the calculated quite closely in all but one instance. Three progenies of the cross Pinto × No. 780 inoculated with race 2 showed a fairly good fit to a 3:1 ratio when the χ^2 value (6.751)[5] was computed for the sum of the values of the individual families. The χ^2 value for the total population of the 3 families was 6.158 [6] (1 degree of freedom), which indicated a rather poor fit to this ratio. The deviations in the 3 families were all minus but nonsignificant. Because of the cumulative effect of these deviations, the total deviation was significant, thus accounting for the high χ^2 value.

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[5] 5-percent point = 7 815.

TABLE 3.—*Reaction of F_2 progenies to physiologic races 1, 2, 6, and 12*

Cross	Progenies	Rust race No.	Plants inoculated: Total	Plants inoculated: Resistant (grade 1)	Plants inoculated: Susceptible (grade 10)	Total χ^2 of individual progenies [1]: Calculated	Total χ^2 of individual progenies [1]: 5 percent point	χ^2 calculated on total of each phenotype in all families [2]
	Number		*Number*	*Number*	*Number*			
Pencil Pod Black Wax × No. 765								
Observed	4	1	264	200	64	0.788	9.488	0.080
Calculated 3:1 ratio				198	66			
Pencil Pod Black Wax × No. 76								
Observed	4	2	593	448	145	1.876	9.488	.095
Calculated 3:1 ratio				444.75	148.25			
Pinto × No. 780								
Observed	3	2	243	199	44	6.751	7.815	6.157
Calculated 3:1 ratio				182.25	60.75			
Cranberry × Pinto								
Observed	2	6	370	278	92	.926	5.991	.004
Calculated 3:1 ratio				277.5	92.5			
Pinto × No. 192.48								
Observed	3	12	465	359	106	2.554	7.815	1.205
Calculated 3:1 ratio				348.75	116.25			

[1] Degrees of freedom = number of progenies.

[2] 1 degree of freedom 5 percent point = 3.841.

TABLE 4.—*Reaction of F_2 progenies to physiologic races 11 and 17*

Cross	Progenies	Rust race No.	Plants inoculated: Total	Plants inoculated: Resistant (grade 1)	Plants inoculated: Intermediate (grade 5–6)	Plants inoculated: Susceptible (grade 10)	Total χ^2 of individual progenies [1]	χ^2 calculated on each phenotype in all families [2]
	Number		*Number*	*Number*	*Number*	*Number*		
Pinto × No. 780								
Observed	3	11	160	40	78	42	3.383	0.150
Calculated 1:2:1 ratio				40	80	40		
Pinto × No. 192.48								
Observed	3	17	461	127	222	112	6.033	1.603
Calculated 1:2:1 ratio				115.25	230.5	115.25		

[1] Degrees of freedom = number of progenies. 5 percent point = 7.815.

[2] 2 degrees of freedom 5 percent point = 5.991.

REACTION TO INDIVIDUAL RACES

RUST RACE 1

Of 44 resistant families (table 5) derived from plants inoculated with rust race 1 in F_2 and tested with the identical race in F_3, 19 were homozygous for resistance and 25 were heterozygous. This is a fairly close fit to a 1:2 ratio with a χ^2 value of 1.920.[7] The segregation of resistant and susceptible plants (fig. 1, *C* and *D*) in the 25 heterozygous families is a good fit to a 3:1 ratio. The total χ^2 values of the individual families is 23.216 [8] for 25 degrees of freedom, and the χ^2 value for the total population of all the families is 2.290 [7] for 1 degree of freedom.

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[6] 5 percent point = 3.841.

The progenies from 10 F_2 selfed susceptible plants were homozygous for susceptibility. These results contribute additional evidence to support the single-factor hypothesis.

RUST RACE 2

Of 54 resistant (fig. 2, *C*) families (table 5) derived from F_2 plants inoculated with rust race 2 and tested with the identical race in F_3, 20 were homozygous for resistance and 34 heterozygous. This segregation is likewise a good fit to a 1.2 ratio with a χ^2 value of 0.218.[7] The 34 heterozygous families segregated in a 3:1 ratio (table 5) The total χ^2 values of the individual families is 44.171 [9] for 34 degrees of freedom. The χ^2 value for the total number of tested plants (1 degree of freedom) is 9.471,[7] which showed a poor fit to a 3:1 ratio. An examination of the data recording the reaction of each individual family but not listed in this paper showed that only 1 family deviated significantly from the calculated. A few others showed fairly large but nonsignificant deviations. The deviations, however, in 25 of the 34 families were in one direction (minus), which accounted, in part, for the high χ^2 value for the total number of plants tested.

Eleven families that were susceptible (fig 2, *D*) in F_2 proved to be homozygous for susceptibility in F_3.

TABLE 5—*Reaction to physiologic races 1, 2, and 6 of F_3 progenies descended from F_2 hybrids inoculated with the respective races*

Physiologic race and classification of plants in F_3 generation	Families tested	Plants inoculated			Total χ^2 of individual families [1]	χ^2 of total of each phenotype in all families [2]
		Total	Resistant	Susceptible		
	Number	*Number*	*Number*	*Number*		
Race 1						
Homozygous resistant (grade 1 2)	19	888	888	0		
Heterozygous resistant (grade 1 2)—						
Observed	25	1,232	947	285	[3] 23 216	2 290
Calculated 3 1 ratio			924	308		
Race 2						
Homozygous resistant (grade 1 2)	20	773	773	0		
Heterozygous resistant (grade 1-2)						
Observed	34	1 567	1 228	339	[4] 44 171	9 471
Calculated 3 1 ratio			1 175 25	391 75		
Race 6						
Homozygous resistant (grade 1 2)	19	598	595	[5] 3		
Heterozygous resistant (grade 1 2)—						
Observed	41	1,844	[6] 1,429	415	[7] 42 824	6 120
Calculated 3 1 ratio			1 383	461		

[1] Degrees of freedom = number of progenies

[2] 1 degree of freedom, 5-percent point = 3 841

[3] 5-percent point = 37 652

[4] Equals 13-percent point.

[5] Probably due to seed mixture

[6] Among resistant plants, 175 severely variegated

[7] Equals 40-percent point

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[7] 5-percent point = 3 841

[8] 5-percent point = 37 652

[9] Equals 13-percent point

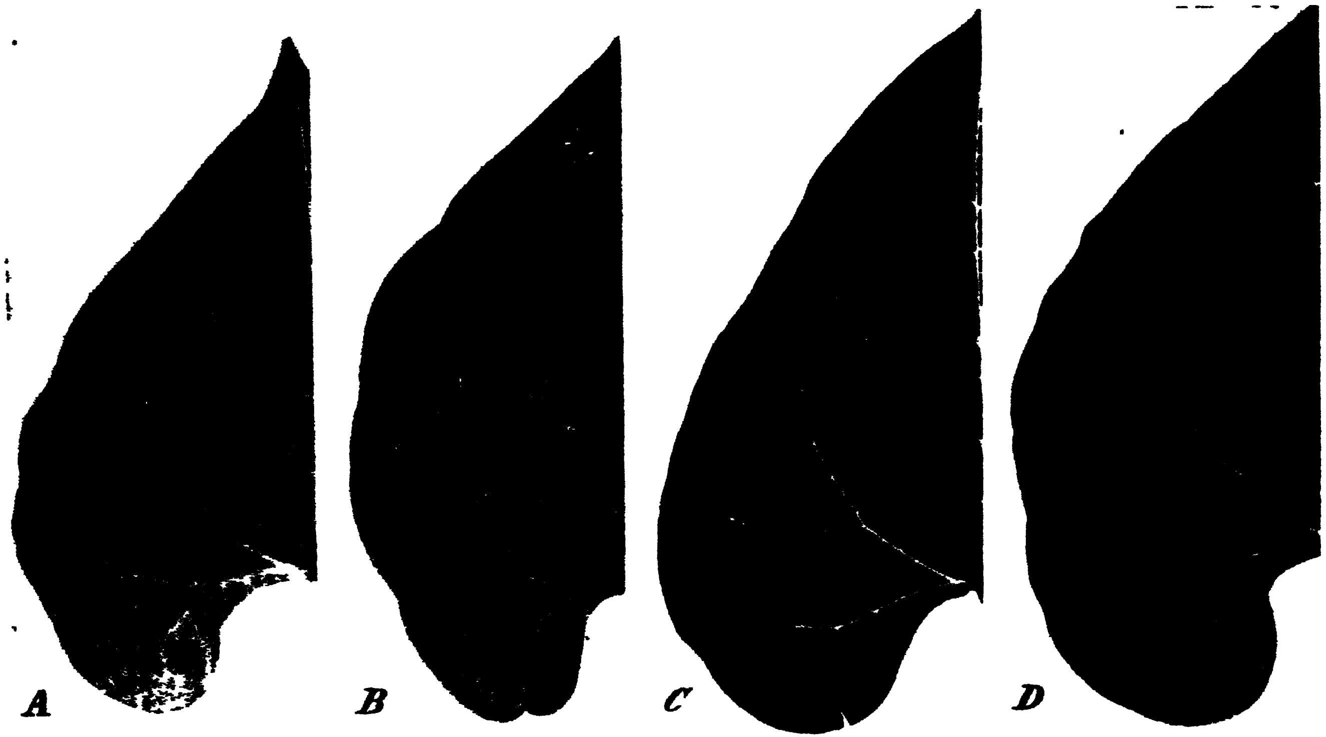

FIGURE 1.—Host reaction to race 1: *A*, Grade 1 on No. 765; *B*, grade 10 on Pencil Pod Black Wax; *C*, grade 1 on F_2 hybrid of cross Pencil Pod Black Wax × No. 765; *D*, grade 10 on another hybrid of same cross.

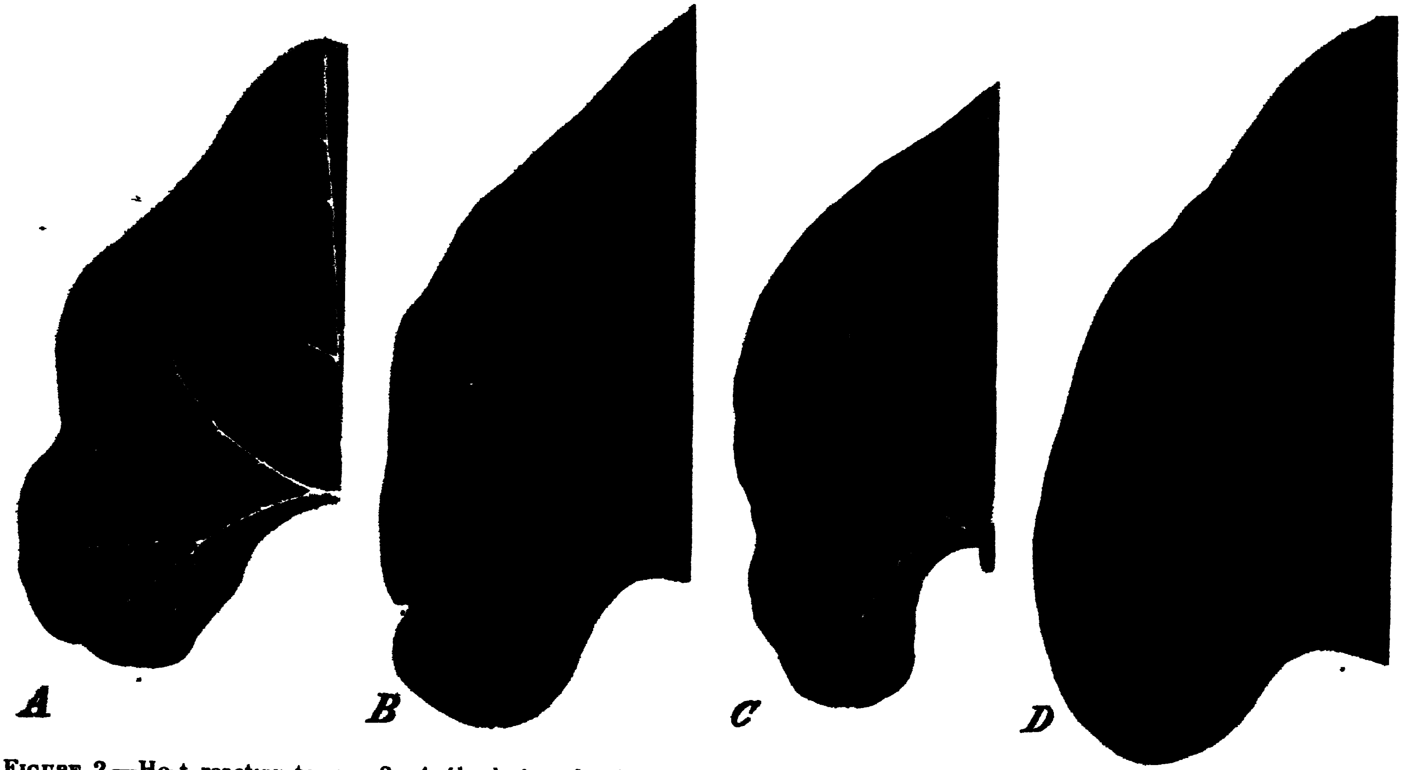

Figure 2—Host reaction to race 2 *A*, Grade 1 on No 780 *B*, grade 10 on Pinto *C*, grade 1 on F_2 hybrid of cross Pinto × No 780 *D*, grade 10 on another hybrid of same cross

Rust Race 6

The F_2 progenies inoculated with race 6 (table 3) segregated in a ratio of 3 resistant plants to 1 susceptible (fig. 3, *C* and *D*) A few plants manifested a leaf variegation (fig 4, *B* and *C*), which was described earlier by the senior writer (*14*) as a heritable abnormality

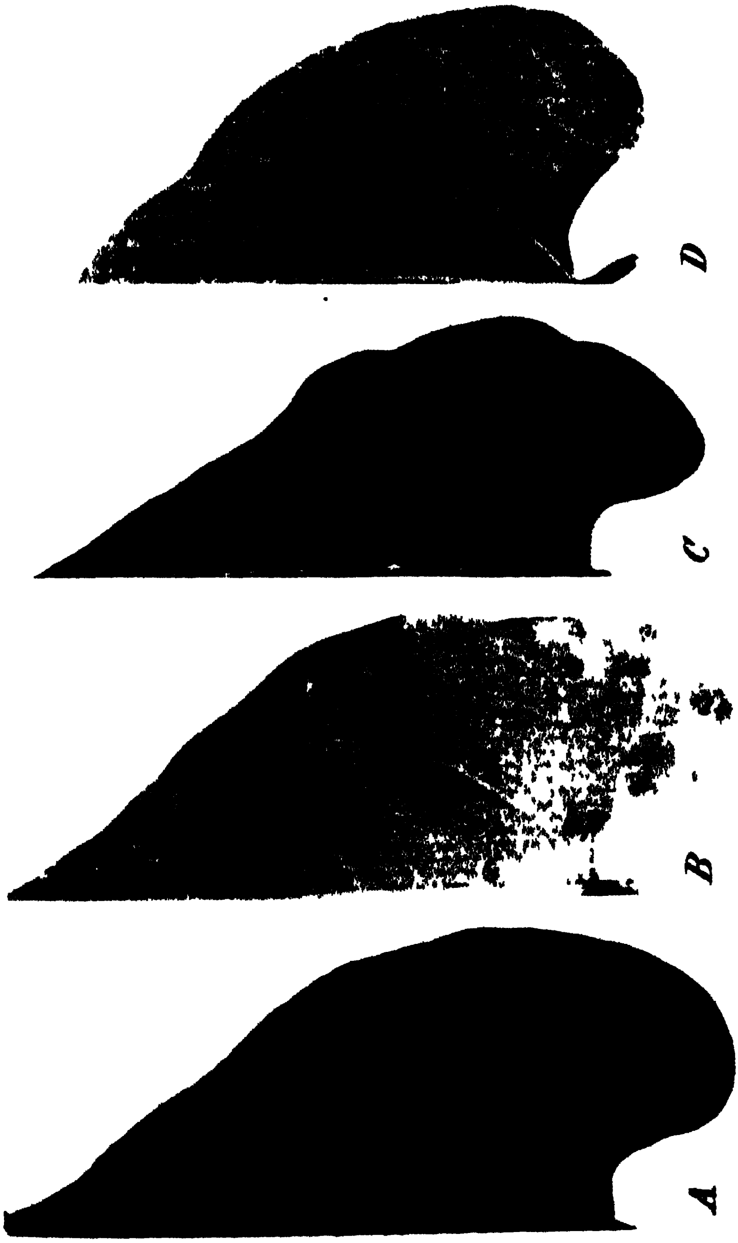

Figure 3 — Host reaction to race 6 *A*, Grade 0 on Cranberry, *B*, grade 10 on Pinto, *C*, grade 0 on F_2 hybrid of cross Cranberry × Pinto, *D*, grade 10 on another hybrid of same cross.

resembling mosaic. The mildly variegated plants showed a subnormal grade of infection (fig. 4, *C*), whereas those severely variegated showed no infection (fig. 4, *B*). Since the number of such plants was small, the ratio of resistance to susceptibility was not greatly altered. In 1 progeny there were 14 variegated plants, 3 of which showed a very serious chlorophyll deficiency; in another, 18 plants were variegated, but none of them were seriously affected.

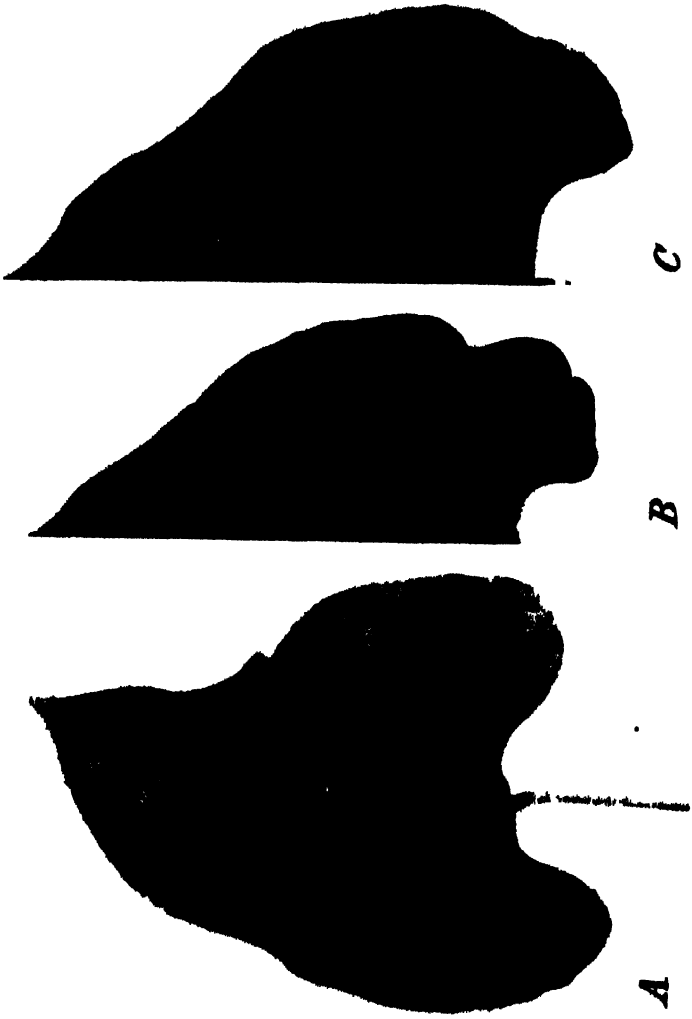

FIGURE 4.—Host reaction of normal and variegated leaves to race 6: *A*, Normal leaf showing abundance of pustules of grades 9 to 10; *B*, grade 0 on seriously variegated leaf; *C*, variegated leaf showing a few pustules of grades 8 to 9

Variegated plants are deficient in chlorophyll, the degree depending upon the extent of the variegation (fig. 4, *B* and *C*). It is likely that the mycelium of a germinating spore enters the host through a stoma, but its progress and development from this point apparently are dependent upon the amount of chlorophyll present in the leaf. In a seriously variegated leaf the fungus possibly dies because of the lack of nutrients. Its progress cannot be great since hypersensitive necrotic lesions seldom occur on such leaves. In moderately or mildly variegated leaves, a few sori were observed but usually only in those regions where some chlorophyll was present. A seriously variegated plant was recorded as resistant even though genotypically it may have lacked the gene responsible for resistance observed in nonvariegated plants.

Of 60 families (table 5) derived from resistant plants inoculated with race 6 in F_2 and inoculated with the same race in F_3, 19 were homozygous for resistance and 41 segregated in a ratio of 3 resistant plants to 1 susceptible. The deviation from the calculated 1:2 ratio of pure-breeding resistant to segregating families derived from F_2 resistant plants was not significant. The χ^2 value is 0.075,[10] which proves a very good fit to this ratio. Among the 19 homozygous resistant families, 3 plants of 598 were susceptible, which possibly resulted from seed mixture.

Of 41 heterozygous resistant families (table 5) with a total population of 1,844 plants, 175 were severely variegated. In many of the progenies where variegation occurred, the number of susceptible plants was slightly below the calculated 3 : 1 ratio. It is not assumed that the deficiency in numbers of susceptible plants was due in all cases to the presence of variegated plants. There was a deficiency, or a —46 deviation, of susceptible plants from the calculated number for a 3 : 1 ratio. The total χ^2 value for this ratio was 42.824 [11] for 41 degrees of freedom, which shows a nonsignificant deviation from a 3 : 1 ratio. The value of χ^2 when computed for the total population of the 41 families was 6.12,[10] which does not indicate a good fit for a 3 . 1 ratio. Twenty-seven of the 41 heterozygous families showed an insufficient number of susceptible plants, which in most cases was due to the presence of variegation. Only 1 family showed a slight significant deviation, but due to the accumulation of deficient numbers of susceptible plants, the χ^2 value of the totals of the tested families fitted a 3 : 1 ratio rather poorly. It is possible that the deviation would have been smaller had there been no variegated plants.

The F_2 susceptible plants did not produce 100-percent susceptibility in F_3. Of a total of 305 plants from 7 families, 22 plants were resistant. All but 1 of these were variegated, from which it is assumed that the lack of true breeding was the result of this chlorophyll deficiency. In 1 family 3 plants were resistant and 2 of these were variegated. The resistant nonvariegated plant may have escaped inoculation or it may have resulted from seed mixture.

The reason for the lack of infection of variegated plants is not definitely known. It may be due to a purely physiologic reaction of the host and fungus or to modifying genetic factors.

The data given above in some respects fail to corroborate those of the F_2 generation, indicating that resistance to physiologic race 6 may not be governed by a single dominant factor. If certain modifying factors are responsible for the lack of true breeding of the variegated plants, more than a single factor may govern the inheritance in the hybrids tested with this race.

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[10] 5-percent point = 3 841

[11] Equals 40-percent point

RUST RACE 12

Thirty-seven families (table 6) derived from the F_2 resistant as well as 17 from the susceptible class were inoculated in the F_3 generation. Fourteen of the 37 families were homozygous for resistance and 23 segregated in a ratio of 3 resistant plants to 1 susceptible. The deviation from the calculated 1 : 2 ratio of homozygous resistant to heterozygous resistant families was nonsignificant with a χ^2 value of 0.34.[12]

TABLE 6.—*Reaction to physiologic race 12 of F_3 progenies descended from F_2 hybrids inoculated with the same race*

Classification in F_3 generation	Families tested	Plants inoculated: Total	Plants inoculated: Resistant	Susceptible: Total	Susceptible: Grade 5	Susceptible: Grade 6-7	Susceptible: Grade 8-9
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Homozygous resistant (0 grade)	14	619	619	0			
Heterozygous resistant							
Observed[1]	23	1,034	[2]772	262	121	44	97
Calculated 3:1 ratio			775.5	258.5			
Homozygous susceptible	17	851	0	851	403	201	247

[1] Total χ^2 of individual progenies was 12.239, 5 percent point = 35.172. χ^2 for total of each phenotype in all families was 0.063, 5 percent point = 3.841.

[2] Resistance grade 0, in 20 families, and grade 3, in 3 families.

The susceptible plants (table 6) in both the heterozygous and homozygous susceptible classes are divided into three grades, i. e., one grade including plants showing extreme susceptibility, approximately as exhibited by the F_2 plants (fig. 5, *D*), and two lower grades. The conditions at the time the F_3 families were inoculated were not as favorable as when the F_2 plants were tested, from which it is assumed that some of this variability may have been due to a lack of favorable environmental conditions for the best development of the rust fungus and host. It is noted (table 6), however, that the segregation of the heterozygous resistant families into 3 resistant plants to 1 susceptible is a good fit to a 3:1 ratio. Three families showed a grade 3 resistance (fig. 5, *E*) and others complete immunity. If the infection had been slightly higher, these three families might possibly have been classed as homozygous susceptible with the assumption that an error had been made in the classification of the F_2 plants. On the other hand, with the extreme difference between a grade 3 rating as shown by 75 percent of the plants in these three families, and a grade 8 to 10 rating as exhibited by 25 percent, it hardly appears likely that any serious error in classification could have occurred.

All of the 17 F_2 homozygous susceptible families were 100 percent susceptible in F_3.

No. 192-48, the resistant parent of the crosses inoculated with race 12 gave a 0 reaction (table 2), whereas in the F_2 the resistant plants gave a grade 1 reaction (fig. 5, *C*). This slight variation may have been due to the environment. On the other hand, the grade 3 reac-

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[12] 5-percent point = 3.841.

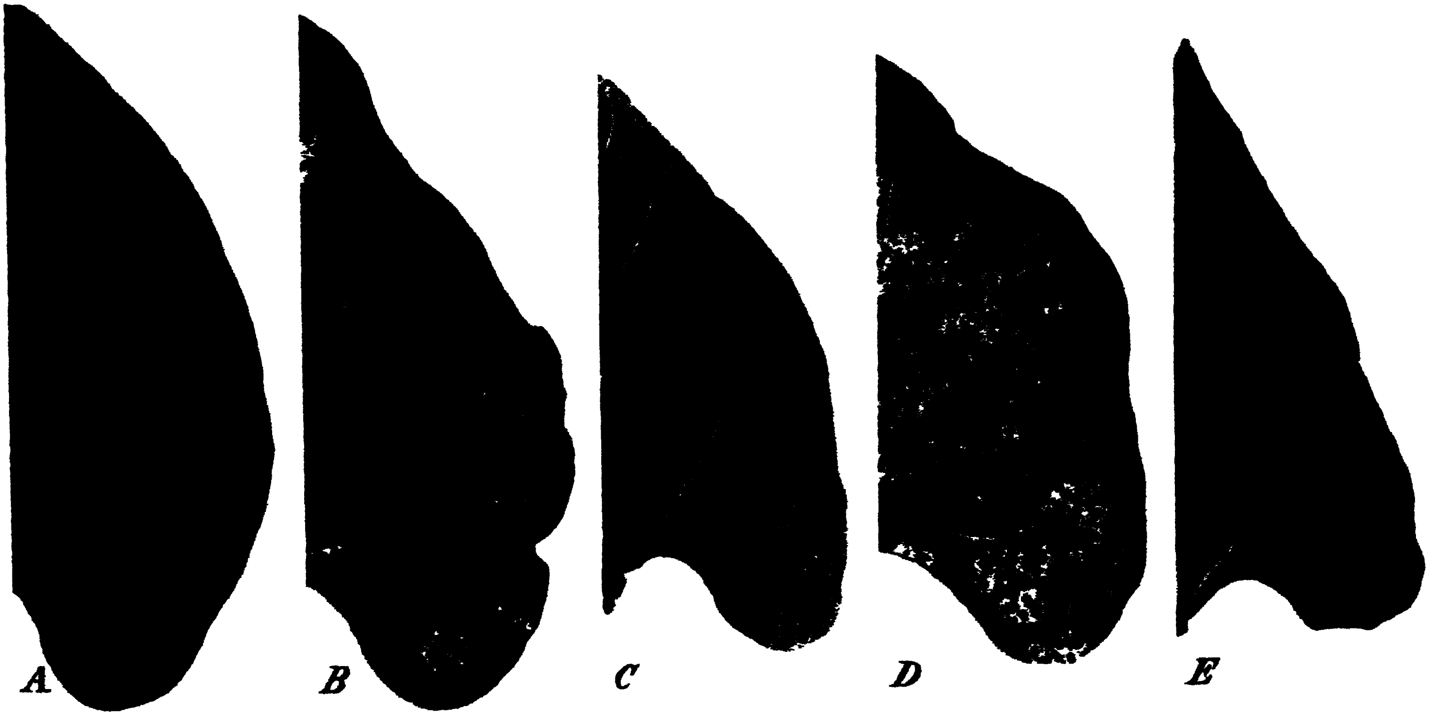

FIGURE 5 —Host reaction to race 12 A, Grade 0 on No 192-48, *B*, grade 10 on Pinto *C*, grade 1 on F_2 hybrid of cross Pinto × No 192-48, *D*, *E*, grades 10 and 3, respectively, on other hybrids of same cross

tion shown by the plants in the above-mentioned three families cannot be explained in this manner. Neither is one justified in assuming that all of the variations in the susceptible class (table 6) were due to environment alone. It is possible that a major genetic factor may govern the range of resistance from 0 to 4, and another, the range of susceptibility from 5 to 10. Clark (*1*), who apparently observed a similar phenomenon in his studies of the inheritance of stem rust and bunt in spring wheat crosses, stated that the effect of minor and modifying factors cannot always be distinguished from the effect of environment. The modifying factors alone or combined with the influence of environment might tend to induce variable degrees of resistance and susceptibility in certain families, but not to the extent that they would be grouped into any except a major resistant or susceptible class. This could account for the facts (1) that some of the heterozygous families did not show the same type of resistance and susceptibility at all times and (2) that the homozygous susceptible families did not exhibit the same degree of susceptibility

In order to determine, if possible, the reason why the F_3 progenies exhibited types of resistance and susceptibility not noted in F_2, another F_2 line of identical parentage was tested and compared simultaneously with several F_3 progenies derived from resistant F_2 plants The inoculations were made under more favorable conditions but not quite as near ideal as when the first F_2 families were tested (table 7)

TABLE 7.—*Comparison of reactions of F_2 progenies with certain F_3 progenies derived from previously tested F_2 plants that were resistant to race 12*

Classification, generation, and progeny No	Plants inoculated Total	Resistant Total	Resistant Grade 0	Resistant Grade 2	Resistant Grade 3 4	Susceptible Total	Susceptible Grade 5	Susceptible Grade 6	Susceptible Grade 8 10	χ² of individual progenies
	No	*No*	*No*	*No*	*No*	*No*	*No*	*No*	*No*	
F_2 progeny, 1229-1										
Observed	171	129	104	19	6	42	20	10	12	[1] 0 017
Calculated 3 1 ratio		128 25				42 75				
Homozygous resistant F_3 progenies										
1227-4-81	80	80	80	0	0	0				
1227-4-85	44	44	0	0	44	0				
Heterozygous resistant F_3 progenies										
1227-4-76	55	42	42	0	0	13	13	0	0	056
1227-4-79	48	35	35	0	0	13	0	0	13	111
1227-4-168	26	16	0	0	16	10	0	0	10	2 513
1227-4-175	40	30	0	0	30	10	0	0	10	0
1227-4-181	30	22	0	22	0	8	0	0	8	044
Total										
Observed	199	145	77	22	46	54	13	0	41	[2] 2 724
Calculated 3 1 ratio		149 25				49 75				

[1] 5-percent point = 3 841

[2] 5-percent point = 11 07 χ² calculated on total of each phenotype in all families was 0 484 for which the 5-percent point was 3 841

The F_2 progeny 1,229-1 (Pinto × No. 192 48) segregated into 3 classes of resistance and 3 of susceptibility (table 7) and showed a good fit to a 3:1 ratio. Two F_3 families were homozygous for resistance, 1 showing total immunity and the other a grade 3 to 4 reaction. Five families with a total of 145 plants of different degrees of resistance plus 54 of several degrees of susceptibility were heterozygous for resistance. The deviation is not significant and the value of χ^2 is 2.724 for 5 degrees of freedom, proving a very good fit to a 3:1 ratio.

The results indicate that actually several types of resistance and susceptibility to race 12 may occur. Why they were not recognized in the F_3 plants inoculated earlier (table 3) is not known, but it is likely that they were masked because of the environment and appeared only under conditions thus far not exactly determined.

RUST RACE 11

The F_2 hybrids inoculated with rust race 11 (table 4) segregated in a ratio of 1 resistant to 2 intermediate to 1 susceptible plant (fig. 6, *C, E, F*). Twenty families in the F_3 generation, derived from previously tested F_2 resistant plants, were inoculated with the same race used in F_2, and all were found to be resistant but not to the same degree as in the F_2 generation. The resistant plants were grouped into four classes (table 8) and in only a few instances did all of the plants in any one family fall into the same class. Twelve progenies fell within either 1 or 2 groups and eight progenies within 4. The progenies that fell within 1 or 2 groups were inoculated under different environmental conditions from those that were grouped into 4 classes.

TABLE 8.—*Reaction to physiologic race 11 of F_3 families descended from F_2 plants inoculated with the same race*

Classification in F_2 generation	Families tested	Plants inoculated: Total	Resistance grade—: 0	Resistance grade—: 1	Resistance grade—: 1–2	Resistance grade—: 2–3	Resistance grade—: 4	Susceptibility grade—: 5–6	Susceptibility grade—: 8–10
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Homozygous resistant (grade 1)	20	1,377	710	469	0	120	78	0	0
Heterozygous resistant (grade 5–6)									
Observed [1]	27	1,769	0	0	452	0	0	891	426
Calculated 1:2:1 ratio					442.2			884.4	442.2
Homozygous susceptible (grade 10)	10	503	[2] 2	0	0	0	0	0	501

[1] Total χ^2 of individual progenies was 22.633 and the 5-percent point was 40.113. The χ^2 calculated for total of each phenotype in all families was 0.859 and the 5-percent point was 5.991.

[2] Possibly the result of seed mixture or of escape from inoculation.

Neither parent (table 2) of the crosses inoculated with race 11 showed a high degree of resistance. No. 780 (fig. 6, *A*), the more tolerant parent, gave a reaction of 6. The F_2 resistant progenies showed a transgressive segregation in that they were more resistant (fig. 6, *C*) than either parent, which would indicate that more than a single factor governed the inheritance of resistance to this race.

If it is assumed that the range of resistance in the F_2 homozygous resistant class could vary no further than from 0 to 2, depending on environmental conditions, it would be difficult to explain on this basis the extreme variability of resistance such as was found in the F_3 homozygous resistant families. Possibly it may be explained according to the theory mentioned earlier with regard to race 12, that is,

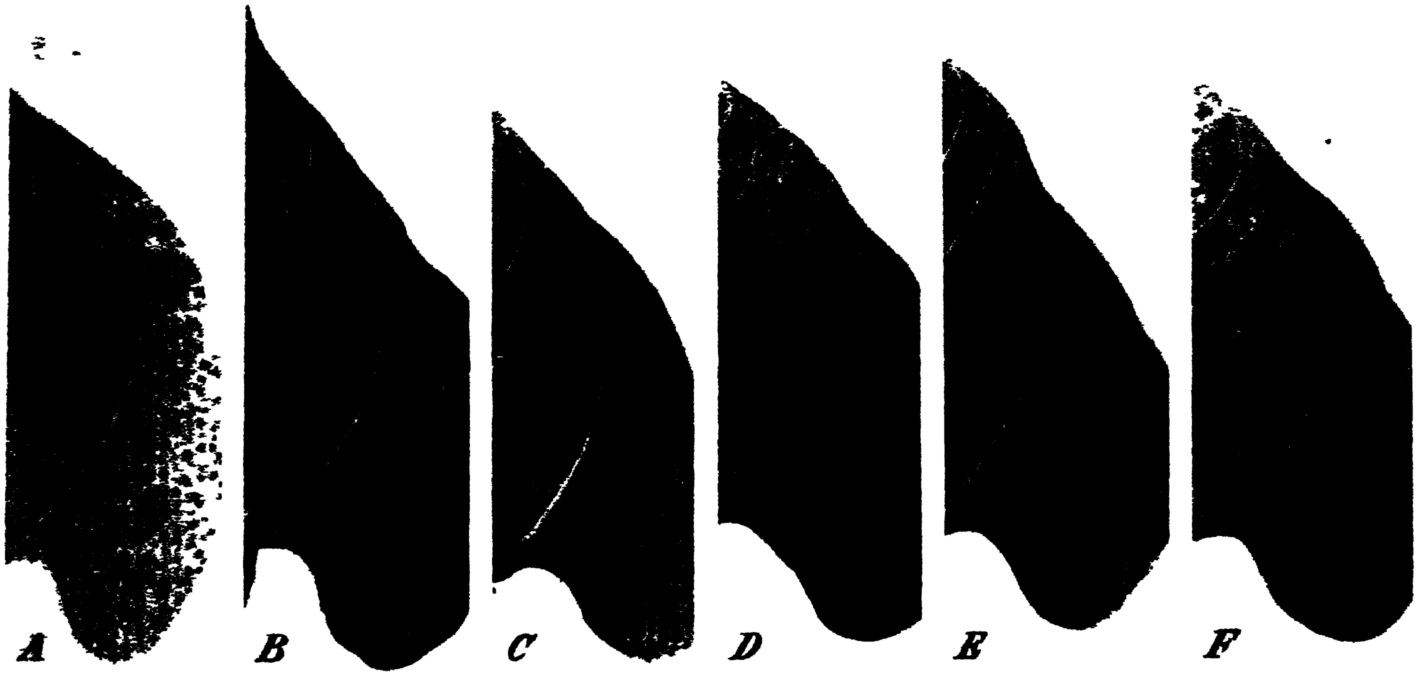

FIGURE 6.—Host reaction to race 11: *A*, Grade 6 on No. 780; *B*, grade 10 on Pinto; *C*, grade 1 on F_2 hybrid of cross Pinto × No. 780; *D*, *E*, *F*, grades 3 to 4, 4 to 5, and 10, respectively, on other hybrids of same cross. Note that necrotic lesions in *C* are larger than in *D* and *E*, but they contained no spores.

that a major dominant gene might be responsible for the range of resistance from 0 to 4 and that minor modifying factors which may differ in amount and effect might be responsible for the other types of resistance shown in the several F_3 homozygous resistant families.

Twenty-seven heterozygous families that showed an intermediate reaction in F_2 segregated in a 1:2:1 ratio in F_3 (table 8), showing that the 3 F_2 generation classes were distinct. The range of resistance was not as variable as it was in the homozygous resistant families, which made it less difficult to separate the plants within a family into their proper classification. Why little, if any, variability occurred in these families as compared with the homozygous resistant families is uncertain. Both classes were inoculated at the same time and under identical environmental conditions. Of 1,769 plants inoculated in the 27 families (table 8), 452 were resistant, 891 intermediate, and 426 susceptible. The χ^2 values shown in table 8 indicate a good fit to a 1:2:1 distribution.

In 10 homozygous susceptible families (table 8) 503 plants were tested and all except 2 were susceptible to approximately the same degree as in the F_2 generation. The 2 resistant plants occurred in separate families, and it is probable that they resulted from seed mixture but they may have escaped inoculation.

A few chlorophyll-deficient plants that differed in appearance from the variegated plants found in the 2 progenies inoculated with rust race 6 were observed in a number of tested F_3 families. These plants, although susceptible to the rust, did not exhibit nearly as high a degree of susceptibility as normal plants. In a few instances they gave a grade 6 reaction, whereas normal plants in the same family gave a grade 10 reaction. In several instances a grade 0 reaction was obtained in plants extremely deficient in chlorophyll. As only a small number of such plants were found in all of the families tested, they were not included in the ratios.

RUST RACE 17

The F_2 hybrids inoculated with rust race 17 segregated in the same manner as those inoculated with race 12, and gave good fits to a 1:2:1 ratio (table 4).

Twelve F_2 homozygous resistant families (table 9) bred true for resistance in F_3. Thirty-nine heterozygous families which showed an intermediate reaction in F_2, segregated into 3 resistant plants to 1 susceptible, although theoretically the segregation should have been identical with that of the F_2, i. e., 1 resistant plant to 2 intermediate to 1 susceptible. The deviation from this ratio was nonsignificant since the value of χ^2 was 0.103, indicating a good fit to a 3:1 ratio. Two grades of resistance (fig. 7, *A* and *B*) were noted (table 9) as well as 2 grades of susceptibility (fig. 7, *C* and *D*). More plants were found in the 0 resistant grade than in grade 2; also, a larger number of plants were found in grade 7 susceptible than in grade 8 to 9.

It is believed that the failure to obtain a 1:2:1 segregation in the heterozygous families was due to a lack of the proper environment for the maximum development of the rust fungus. Only 86 of the 387 susceptible plants in the heterozygous resistant families (table 9) gave a grade 8 to 9 reaction instead of a grade 10 as they did in the F_2 (table 4). The same is true in the homozygous susceptible families, where of a total of 897 plants, only 342 were rated grade 8 to 9 in susceptibility. It is assumed that if conditions had been optimum approximately two-thirds of the resistant plants in the heterozygous families would have given an intermediate reaction, substantiating the 1:2:1 ratio of the F_2. Instead, these plants fell within the resistant class, making a 3:1 ratio of resistance to susceptibility. The

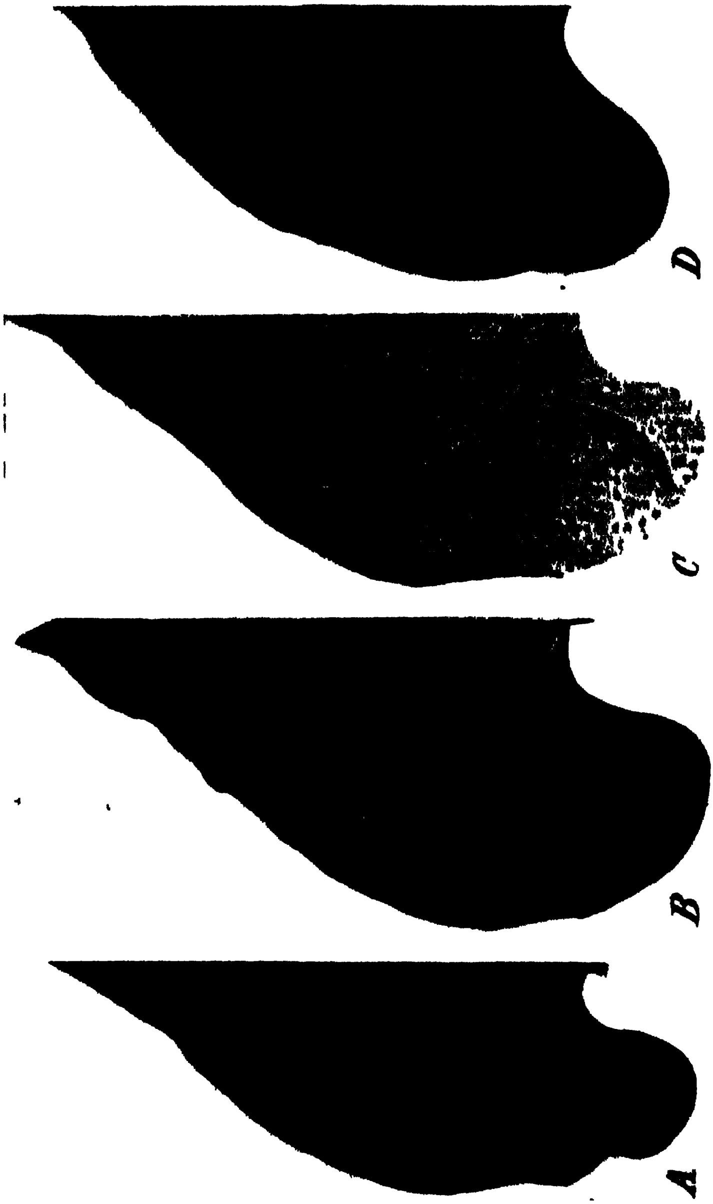

FIGURE 7.—Host reaction to race 17. *A*, Grade 0 on hybrid of cross Pinto × No. 192-48; *B*, *C*, *D*, grades 2, 7, and 10, respectively, on other hybrids of the same cross.

23 homozygous susceptible families bred true for susceptibility, but the plants fell within 2 classes (table 9).

TABLE 9. *Reaction to physiologic race 17 of F_3 families descended from F_2 plants that had been inoculated with the same race*

Classification in F_2 generation	Families tested	Plants inoculated						
		Total	Resistant			Susceptible		
			Total [1]	Grade 0	Grade 2	Total	Grade 7	Grade 8 9
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Homozygous resistant (grade 1)	12	409	409	409	0	0	0	0
Heterozygous intermediate (grade 5 6)								
Observed	39	1,570	1,183	761	422	387	301	86
Calculated 3 1 ratio			1,177 5			392 5		
Homozygous susceptible (grade 10)	23	897	0	0	0	897	555	342

[1] Total χ^2 of individual families was 29 923, which = 15-percent point χ^2 calculated on total of each phenotype in all families = 0 103 5 percent point = 3 841

The F_3 inoculations were conducted at two different periods during the winter of 1939 40. Environmental conditions were less favorable when the first set was made than when the second set was made. The results, especially in the resistant class of the heterozygous families, clearly indicated this difference. In the first inoculations the results showed that of a total of 640 resistant plants, 625 fell within grade 0 and 15 within grade 2. Of a total of 543 plants, in the second inoculations, 136 fell within grade 0 and 407 within grade 2.

To study further the reason that the intermediate F_2 class failed to segregate in a 1:2:1 ratio in F_3, 2 untested F_2 families and 11 more F_3 families from tested F_2 lines that had produced an intermediate reaction were inoculated with race 17. The results are shown in table 10. The environmental conditions were slightly more favorable than when F_3 plants were tested earlier (table 9); however they were not as favorable as when the first F_2 families were inoculated (table 4). The data presented in table 10 do not appear to be sufficiently consistent to be interpreted on the basis of a one-factor pair difference. In progeny 1228–4, the intermediate class was slightly more resistant than the F_2 progenies listed in table 4. Two grades of susceptibility were produced, whereas in the earlier tested F_2 plants only a susceptible 10 grade was noted. On the other hand, if the plants in grade 3 are regarded as resistant F_2 progeny 1228–5 segregated in a 3:1 ratio. It is likely that the plants in grade 3 might have shown an intermediate reaction under a more favorable environment. Furthermore, it is possible that part of the plants in the 0 grade may have been genotypically intermediate in reaction but failed to reach that degree of development because of the unfavorable environment. On the other hand, environment alone may not have been responsible for the variability, but the reaction may have been influenced by certain modifying factors.

TABLE 10.—*Reaction of F_2 progenies and F_3 progenies descended from previously tested F_2 plants which had shown an intermediate reaction (grade 5–6) to physiologic race 17*

Generation and family No.	Families tested	Plants inoculated								Total χ^2 value of individual families[1]	
		Total	Resistant	Intermediate resistant			Susceptible			Calculated	5-percent point
				Total	Grade 3	Grade 4	Total	Grade 7-8	Grade 9		
F_2	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*		
1228-4	1	113	31	48	0	48	34	13	21	2.722	5.991
1228-5	1	200	83	69	69	0	48	31	17	107	3.841
F_3											
Observed	11	497	132	254	254	0	111	96	15	[2]14.278	33.924
Calculated 1:2:1 ratio			124.25	248.5			124.25				

[1] Degrees of freedom = twice the number of families tested except for family 1228-5.

[2] χ^2 calculated on totals of each phenotype in all families = 2.018; 5-percent point = 5.991.

Four hundred ninety-seven F_3 plants derived from previously tested F_2 lines that had given an intermediate reaction were inoculated (table 10) with the result that 132 were found to be resistant, 254 intermediate, and 111 susceptible. If the plants in grade 3 are considered as intermediate in reaction then the total χ^2 value of the individual families for a 1:2:1 distribution is 14.278,[13] which shows a good fit for this ratio. The χ^2 value for the total population (2.023) also proves a good fit. The intermediate class showed a higher degree of resistance than did the earlier tested F_2 plants (table 4), which was assumed to be the result of the environment. In the previously tested F_3 progenies (table 9) a considerable number of plants in the families that gave an intermediate reaction in F_2 showed a grade 2 reaction. Many of these may have given an intermediate reaction under more favorable environmental conditions. Classification was difficult because of the similarity between these plants and those in the 0 class.

The susceptible plants were divided into 2 groups, the majority of which fell within the 7 to 8 grade. Many, if not all, of these plants might have attained a higher degree of infection had conditions been more favorable. On the other hand, minor or modifying factors may also have been responsible for certain of the plants not attaining the degree of infection that was shown by the F_2 plants. These same factors may have been the cause of the failure of the F_2 intermediate class of previous experiments to segregate in the expected 1:2:1 ratio in F_3.

DISCUSSION

It is evident from the data presented in this study that in the bean hybrids investigated a single genetic factor is involved in the inheritance of resistance to physiologic races 1 and 2 of bean rust, and that more than one factor may govern such inheritance to races 6, 11, 12, and 17. It is also shown that the factor for resistance is dominant in the hybrids inoculated with races 1, 2, 6, and 12, and incompletely dominant in the crosses inoculated with races 11 and 17.

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[13] 5-percent point = 33.924

No. 780, the less susceptible of the two parents of the crosses inoculated with race 11, exhibited (table 2) a grade 6 reaction (fig. 6, *A*), whereas Pinto, the more susceptible parent, showed a grade 10 reaction (fig. 6, *B*). In the F_2 generation, the plants in the homozygous resistant class were less susceptible (fig. 6, *C*) than No. 780, thereby indicating a transgressive segregation.

The inheritance of resistance to races 6, 11, 12, and 17, as previously mentioned, may be governed by more than a single gene even though the F_2 results indicate a single-factor difference. Although it is possible that the lack of infection among the chlorophyll-deficient plants inoculated with race 6 could be explained on a purely physiological basis, it is also likely that this phenomenon may have been due to minor or modifying factors.

An F_2 progeny closely related to those that were earlier inoculated with race 12 was later inoculated with the same race and a wide range of resistance and susceptibility was noted (table 7), although only one type of resistance was observed in the first test. All of the homozygous resistant F_2 plants inoculated with race 12, as well as the resistant plants of the heterozygous resistant families, did not breed true for the same type of resistance as shown in the F_2 plants (table 3) but exhibited a greater range of resistance. Similarly, the homozygous susceptible F_2 plants did not breed true in every case in the F_3 for the type of susceptibility exhibited in F_2 (tables 3 and 6). Likewise, not all of the plants in the homozygous resistant families inoculated with race 11 bred true for the same type of resistance as shown by the F_2 plants (table 4). Some but not all of this variation may have been caused by environment, and minor modifying factors may have been responsible to some extent. Why these types of resistance and susceptibility were not recognized in the earlier inoculated F_2 plants is difficult to explain. It is possible that an exacting environment not thus far recognized is essential for their production. There is likewise a possibility that a major gene may inhibit their production under most conditions.

The two grades of susceptibility recognized among the susceptible plants as well as the variability in the plants showing an intermediate reaction when inoculated with race 17 (tables 9 and 10) probably resulted from a combination of modifying genetic factors and environmental influences.

These studies showed that environment probably played an important role, as regards its influence both on the host and on the fungus, and this may explain, in part at least, why many of the readings taken under one set of conditions differed from those taken under another.

Hybrids inoculated with races 11 and 17, gave in F_2 (table 4) a ratio of 1 resistant plant to 2 intermediate to 1 susceptible. The plants that showed an intermediate reaction to race 11 in F_2 segregated in a similar manner in F_3 (table 8), whereas the plants in the same class inoculated with race 17 under the same environmental conditions segregated in 3 resistant plants to 1 susceptible (table 9). When the inoculations with race 17 were made under more favorable conditions, the intermediate class segregated in a 1:2:1 ratio in F_3 (table 10) as expected. The intermediate class, however, shifted from a grade

5 to 6 rating (table 4) to a grade 3 (table 10), and the susceptible class from a grade 10 rating (table 4) to grades 7 to 8 and 9 (table 10). This suggests that because of the unfavorable environment those plants that should have shown an intermediate reaction in the earlier F_3 tests appeared quite highly resistant and were classified with the homozygous resistant plants. The combination of these two classes compared with the homozygous susceptible class gave a 3:1 ratio, which indicates that the environment may definitely affect the results and may play a more important role with some races than with others.

These data have convinced the writers that certain uncontrollable differences in environment, even though slight, may be sufficient to shift the degree of infection into lower grades than would normally be expected. It was not deemed advisable to adhere at all times to an arbitrary standard of classification. It has been necessary to take into account all the factors that were known to influence the results before assigning infected material to the different classes. The writers believe that these records of actual occurrence may be of benefit to other investigators who may note similar phenomena in their experiments.

It is not known how many of the 11 grades used in classifying the degree of resistance and susceptibility may be governed by a single gene. Three general classes have been recognized, that is, resistant, intermediate, and susceptible, and within these other subclasses were noted.

It should be remembered that the results presented herein deal only with the inheritance of resistance in the seedling stages of growth under greenhouse conditions. Earlier studies by Stakman (*12*), Melchers and Parker (*10*), and Hayes, Stakman, and Aamodt (*8*) with the stem rust of wheat showed that certain varieties and hybrids of wheat in the seedling stage showed relatively high susceptibility under greenhouse conditions but considerable resistance as adult plants in the field. It was also shown by Goulden, Neatby, and Welsh (*4*) that resistance in the mature stage was inherited independently of seedling resistance, and they concluded that the two types of resistance are distinct. Levine and Smith (*9*), working with races of oat rust (*Puccinia graminis avenae* Eriks.), found close agreement in the reactions of seedling and maturing plants. This phase of the bean rust problem has not been investigated, but observations have indicated that many varieties susceptible in the maturing stages in the field are equally as susceptible in the seedling stage in the greenhouse

At the present time relatively few commercial varieties of either the garden or the field types are resistant to many of the races However, there are a few that have shown a high degree of resistance to a number of the races thus far described. The writers have employed some of these varieties in certain crosses, and a number of hybrids resistant to 17 races are now being developed.

SUMMARY

Four different crosses involving six varieties or strains of beans and six physiologic races of bean rust were investigated to determine the mode of inheritance of resistance. The races of rust involved were 1, 2, 6, 11, 12, and 17.

Results proved that resistance to races 1 and 2 in the bean hybrids investigated was due to a single Mendelian factor. Possibly other genetic factors may be involved in the resistance of the hybrids inoculated with races 6, 11, 12, and 17. Resistance was shown to be dominant in the hybrids inoculated with races 1, 2, 6, and 12, and incompletely dominant in those inoculated with races 11 and 17.

Severely variegated plants inoculated with race 6 showed immunity, whereas those mildly variegated showed a lesser degree of susceptibility than normal plants. This may have been due either to the physiological behavior of the host and the fungus, or to modifying genetic factors, or to a combination of these.

It is possible that a major gene may govern resistance within grade 0 to 4 in the hybrids inoculated with races 11 and 12, and a similar factor susceptibility within grade 5 to 10. Minor modifying factors may be responsible for the variable degrees of resistance and susceptibility found within the major classes.

An F_2 line related to those progenies previously inoculated with race 11 showed several classes of resistance and susceptibility not previously observed, which explains in part at least the reason for the presence of these variable classes in F_3.

Transgressive segregation occurred in the hybrids inoculated with race 11 since one-fourth of the F_2 plants showed more resistance than the less susceptible parent.

The results with the F_3 progenies inoculated with race 17 showed that environment exercised some influence on the degree of infection in the intermediate class. Under an unfavorable environment the plants appeared resistant and segregated in a ratio of 3 resistant to 1 susceptible, whereas under more favorable conditions they segregated in a 1:2:1 ratio.

These data deal only with the inheritance of resistance of seedling plants grown under greenhouse conditions.

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IODINE IN RELATION TO PLANT NUTRITION[1]

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INTRODUCTION

Although investigations dealing with the effects of iodine on plant growth have been reported over a period of some 40 years, the exact relation of iodine to plant nutrition remains to be determined. Interest in this problem is augmented by the importance of iodine in animal nutrition.

The writers' approach to the problem of the importance of iodine in plant nutrition has involved two distinct lines of study. First of all, critical experiments dealing with the question of the essential nature of iodine in plant nutrition have been conducted. The knowledge as to whether occasional stimulations of plant growth by iodine additions to soils are due to the remedying of a deficiency of an essential nutrient or to some secondary effect of iodine is of prime importance to a rational consideration of the problem. A second line of approach has been based on the assumption that responses to iodine additions to soils are most likely to be found in regions where simple goiter is endemic. Oregon is in such a region (*5*).[3]

REVIEW OF LITERATURE

Several investigators (*14*, *21*, *22*)[4] have obtained stimulations of plant growth from additions of iodine to sand and solution cultures. White (*23*) found that iodine, as well as manganese, zinc, and boron, appears to be essential for the growth of excised tomato roots. Numerous failures to obtain stimulations by nontoxic additions of iodine-bearing compounds to sand and solution cultures have also been reported (*4*, *16*, *19*). Iodine levels of the order of 1 or 2 parts per million are generally toxic to higher plants, except halophytes.

Growth responses of micro-organisms to iodine treatments have also been reported. Several workers have reported stimulation of *Azotobacter chroococcum* (*6*, *10*, *21*), *Saccharomyces cerevisiae* (*7*, *10*), and *Bacillus subtilis* (*10*) by iodine treatments. Ishikawa (*9*), however, found that the activity of proteolytic bacteria was repressed by as little as 20 p. p. m. of iodine. These effects were obtained with

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[2] The authors gratefully acknowledge the cooperation of Dr. J. R. Haag in furnishing facilities for iodine determinations, and of Dr. W. B. Bollen and Dr. C. P. Hegarty in furnishing facilities for the microbiological work.

[3] Italic numbers in parentheses refer to Literature Cited, p. 636.

[4] REGEIMBAL, L. O. THE ROLE OF IODINE IN PLANT NUTRITION. 50 pp. Minneapolis, Minn. 1929. (Thesis, Minn. Univ)

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iodine concentrations well below the toxic levels, since for microorganisms relatively high concentrations are required to produce toxic effects. Such growth responses are to be distinguished from the stimulations frequently given by poisons at concentrations just below toxic levels. Thus Branham (*1*) investigated 16 antiseptics, including tincture of iodine, and found that most of them had a stimulating effect at sublethal concentrations. The latter phenomenon is related to the irritability of protoplasm and must be carefully distinguished when the question of the essentiality of a nutritive factor arises.

Since even in solution cultures the reasons for growth responses to iodine additions are not clear, it is not possible at present to interpret satisfactorily the responses sometimes obtained when iodine is added to soils. Numerous cases of growth responses are recorded. Not all of these experiments may be dismissed as being statistically unreliable. As would be expected, there are also numerous reports of failures to obtain responses. The effects of iodine treatment of soils on plant growth and on the iodine content of plants, as well as the distribution of iodine in nature, have been reviewed by McClendon (*15*) and others (*13*, *17*).[5]

THE QUESTION OF THE ESSENTIAL NATURE OF IODINE IN PLANT AND MICROBIOLOGICAL NUTRITION

MATERIALS AND METHODS

Analytical Methods

The analytical methods employed varied with the type of material to be analyzed and the anticipated iodine content. Water samples were evaporated under alkaline conditions, followed by low temperature potassium hydroxide fusion. Soils and high-iodine plant materials were fused with potassium hydroxide. Low-iodine plant materials were burned in the Kolnitz-Remington (*11*) oxygen torch. The gases were absorbed in alkaline solution, the solution evaporated, and the residue fused with potassium hydroxide. Iodine was recovered by the distillation method of Bratton and McClendon (*2*), followed by bromine oxidation and thiosulfate titration. In some of the earlier analyses, iodine was recovered by alcohol extraction of a potassium carbonate paste (*8*) or a saturated solution of potassium carbonate (*18*).

Numerous blank determinations were run as a precaution against the ever-present danger of iodine contamination. The results from these blanks were usually small in comparison with the actual determinations. The recovery of iodide added to hay samples was found to vary from 90 to 100 percent. The analytical figures presented are usually the average of two or more determinations.

Purification of Nutrient Chemicals

The only report found in the literature of a cultural medium especially designed to contain a minimal amount of iodine is that of Stoklasa (*22*). He gives no details of his purification methods beyond the implication that the nutrient salts were purified by recrystallization. Stoklasa's analyses of plant materials grown on his medium show a substantial iodine content. The writers' analyses, presented in table 1, emphasize the necessity for purifying certain of the nutrient chemicals.

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[5] Karns, G. M. the relation of iodine to plant growth. 13 pp. Pittsburgh. (Mimeographed report of the Mellon Institute of Industrial Research.)

Since little evidence was available as to the efficiency of removal of iodine compounds from nutrient salts by recrystallization, more positive purification methods were sought for use with the necessary nutrient chemicals. Where possible, the maximal iodine content of the chemical after purification was approximated by studying the rate of removal of iodine during the purification procedure. Thus in the purification of dipotassium phosphate, a saturated solution of the salt was repeatedly extracted with ethanol The progress of iodine removal was followed by analyses of the successive alcohol extracts.

It is estimated that the procedures followed are sufficiently effective to reduce the iodine content of the major nutrient chemicals to below 1 part per billion; that of water to below 0.001 p. p. b. The resulting nutrient solutions should have an iodine content of the order of 0.001 p. p. b. Such chemicals and solutions are hereafter referred to as "iodine-free."

TABLE 1 *The approximate iodine content of some chemically pure chemicals and other substances*

Chemical	Iodine content	Chemical	Iodine content
	Parts per billion		*Parts per billion*
Dipotassium phosphate	25	Magnesium sulfate	18
Monopotassium phosphate	16	Ferric tartrate	100
Phosphoric acid	2.5	Ammonium hydroxide, on the basis of NH_3 content	5
Potassium carbonate	20	Sucrose, household	< 3
Potassium hydroxide	15	Starch, household	15
Potassium sulfate	25	Acetone	8
Potassium nitrate	5,000	Ethanol	2
Sodium nitrate	200	Carbon tetrachloride	< 1
Sulfuric acid	< 2	Carbon dioxide, compressed technical grade, on the weight basis	< 2
Calcium sulfate	140	Water, Corvallis tap	.35
Calcium hydroxide, technical grade	50	Water, laboratory-distilled	.005
Calcium carbonate	1,000	Water, redistilled from alkali carbonates	< .001
Calcium lactate	1,000		

Dipotassium phosphate and *potassium carbonate*.—A saturated solution of the salt was extracted repeatedly with iodine-free ethanol prepared as described below. When analyses of the extracts showed a reduction of the iodine content of the salt to less than 1 p. p. b., the solution was boiled for 30 minutes with 0.1 percent of hydrazine sulfate to reduce iodates. After reextraction with ethanol, the solution was evaporated to dryness and heated to destroy excess hydrazine.

Sulfuric acid.—The acid was boiled to about two-thirds of the original volume.

Calcium sulfate, *magnesium sulfate*, and *ferric sulfate*.—The finely divided salt was suspended in two times its volume of chemically pure sulfuric acid and boiled to dryness.

Ammonium hydroxide.—Ammonia was purified by distillation from potassium carbonate, bubbling through potassium carbonate solution in a series of gas-washing bottles, and absorption in iodine-free water.

Nitric acid.—Chemically pure nitric acid was repeatedly distilled from barium nitrate, only the midfraction being saved. Acid several years old was used to ensure complete conversion of iodides to iodates. Owing to the difficulty of determining traces of iodine in the presence of large amounts of nitrates, this purification procedure could not be critically checked.

Sucrose.—The method of Harvey (*8*) involving repeated recrystallizations from ethanol was followed.

Ethanol.—Ethanol was readily freed from iodine compounds by distillation from potassium hydroxide after an initial refluxing period. The ethanol used for cultural purposes was redistilled.

Water.— Purification of ordinary distilled water was accomplished by redistillation from alkali carbonates in a pyrex glass still.

CULTURAL METHODS

Two groups of experiments were conducted with higher plants. The culture solutions used in the first group were prepared from ordinary chemically pure salts and ordinary distilled water; only nominal precautions were taken against iodine contamination. These experiments are referred to hereafter as "preliminary." The solutions used in the second group of experiments were prepared from the specially purified salts and redistilled water, and rigorous precautions were taken against iodine contamination.

The basal nutrient solution used in the preliminary experiments and the cultural conditions have been described elsewhere (*12*). In all of the cultural work iodine treatments were added as potassium iodide. Stoneware and mason jars were used as cultural vessels. The seedlings, after germination in washed quartz sand, were transferred to the cultural solutions. They were held with tufts of cotton in varnished plywood covers.

The nutrient solutions used in the second group of experiments were prepared from iodine-free nutrient chemicals and redistilled water. The chemically pure salts used for supplying minor elements were not subjected to purification procedures. The nutrient solutions had the following composition: Calcium sulfate, 0.002 M; magnesium sulfate, 0.001 M; dipotassium phosphate, 0.002 M; ammonium nitrate, 0.005 M; boron as boric acid, 0.5 p. p. m.; manganese as sulfate, 0.5 p. p. m.; zinc as sulfate, 0.05 p. p. m.; copper as sulfate, 0.02 p. p. m.; and molybdenum as ammonium molybdate, 0.05 p. p. m. The pH was adjusted weekly to 5.5 to 6.0 with ammonium hydroxide or nitric acid. Iron was added as ferric sulfate at the rate of 1 p. p. m. every 3 days during the early growth stages and weekly during the later growth stages.

The success of such experiments depends on careful precautions against contamination of the reagents, nutrient solutions, and plants. In the case of iodine this is especially important because of its wide distribution. The glass culture vessels, glass-stoppered bottles used for storing reagents, and pipettes and graduated cylinders used in preparing the solutions were thoroughly soaked in acid cleaning mixtures and rinsed well with iodine-free water. The interference of iodine contamination of the solutions was reduced as far as possible by avoiding forced aeration and by using concentrated, infrequently renewed culture solutions.

The medium used for the culture of *Azotobacter agile* was prepared from iodine-free salts, and precautions were taken against iodine contamination. The medium had the following composition: Magnesium sulfate, 0.0084 M; dipotassium phosphate, 0.0019 M; calcium sulfate, 12 p. p. m.; ferric sulfate, 5 p. p. m.; boron, manganese, zinc, copper, and molybdenum as given above; potassium carbonate to pH 7.0; and ethanol, 1.1 percent by weight. The inorganic constituents were dissolved in iodine-free water. Aliquots of 140 ml. were distributed into 500 ml. Erlenmeyer flasks, which were stoppered with cotton and autoclaved. Ethanol was aseptically pipetted into the flasks after sterilization. The inverted-sucrose medium used in the preliminary experiments had a similar inorganic composition.

EXPERIMENTAL RESULTS

PRELIMINARY PLANT EXPERIMENTS

A number of preliminary experiments were conducted with ordinary c. p. chemicals and tap or distilled water to determine the effect of added iodine. The results obtained in certain of these experiments are reported in table 2.

TABLE 2.—*Yield and iodine content of corn, barley, and head lettuce grown in unpurified solution cultures containing small quantities of iodine*

Iodine added to solution (p. p. m.)	Corn			Barley			Head lettuce		
	Mean dry weight of tops at 60 days	Iodine content, dry basis	Fraction of added iodine absorbed	Mean green weight at 60 days	Iodine content,[1] dry basis	Fraction of added iodine absorbed	Mean green weight at 60 days	Iodine content, dry basis	Fraction of added iodine absorbed
	Grams	*P. p. m.*	*Percent*	*Grams*	*P. p. m.*	*Percent*	*Grams*	*P. p. m.*	*Percent*
0.0	17.2±2.0	0.05		26.8±2.5	0.20		44.4±2.1	[1]0.27	-
0.05				24.0±3.5	15	24	51.8±7.6		
0.10	14.0±1.8	8.6	11	23.8±2.1	16	12	49.9±6.6	8.7	56
0.25				[3]25.9	90	32	59.1±7.0	18	58
0.50	11.8±1.8	58	13	20.8	160	21	55.1±4.7	39	59
1	9.9±1.5	88	8	(4)			38.7±4.0	65	31
5	2.2±0.4	430	2						

[1] Duplicate analyses were not made because of insufficient plant material.
[2] Mean of 3 living plants, 3 plants dead.
[3] Mean of 5 living plants, 1 plant dead.
[4] All plants dead.

Additions of potassium iodide equivalent to 0.1 to 5 p. p. m. of iodine depressed the growth of corn (*Zea mays*). A concentration of 1 p. p. m. of iodine depressed growth by approximately 50 percent, while 5 p. p. m. was very toxic. The above culture solutions were made up with tap water in new glazed stoneware crocks.

In a similar experiment with barley (*Hordeum vulgare*), in which solutions made up with distilled water in glazed stoneware crocks were used, no stimulation was observed over the range of 0.05 to 1 p. p. m. of added iodine. A concentration of 0.5 p. p. m. of added iodine was definitely toxic; 1 p. p. m. was lethal.

Another experiment with barley was made to determine the statistical significance of the results from the addition of 0.05 p. p. m. of iodine. The cultural solutions were prepared with distilled water. Eighteen

1-quart mason jars were used for each treatment. The mean dry weight of the tops was 0.422 ± 0.033 gm.[6] for the control solution as compared with 0.457 ± 0.025 gm. when 0.05 p. p. m. of iodine was added. The difference is not statistically significant.

An experiment was made with head lettuce (*Lactuca sativa*), grown in glazed stoneware crocks in solutions made up with distilled water. Iodine was added at rates of 0.05 to 1 p. p. m. (table 2). The control plants were inferior to those treated with 0 05 to 0.5 p. p. m. of added iodine. The plants treated with 1 p p. m. of iodine were slightly inferior to the controls. While the responses to individual iodine treatments are not statistically significant, if the iodine treatments from 0.05 to 0.5 p. p. m. are grouped, a slight response to added iodine appears to have been obtained. The control treatment was applied to six plants, the other treatment to four plants each.

In another experiment, head lettuce was grown in 1-quart mason jars in solutions made up with distilled water. A treatment with 0.25 p. p. m. of added iodine was compared with the control solution. No stimulation was observed. Further work with head lettuce in stoneware jars failed to show any stimulation resulting from the added iodine.

TABLE 3 — *The calculated iodine content of the control solutions used by several investigators*

Workers	Plant analyzed	Average iodine content of the control plants	Average calculated iodine content of the solution	Independent estimates	Remarks
		P. p. b.	*P. p. b.*	*Number*	
Stoklasa (*21*)	Day lily (*Hemerocallis fulva*)	120	2 500	4	
Stoklasa (*2*)[2]	Sugar beet				Purified chemicals and ignited sand culture
	Tops	640	1 000	5	
	Roots	210	2 700	5	
Scharrer and Schropp (*19*)	Sugar beet				Sand cultures
	Tops	1 370	25	7	
	Roots	670	55	7	
	Turnip				
	Tops	970	55	6	
	Roots	1 150	80	6	
Orr, Kelley, and Stuart (*16*)	Peas (1)	[1] 140	35	3	Reagent grade chemicals
	Peas (2)	[1] 81	35	1	
Regeimbal	Wheat				Purified chemicals
	Tops	190	50	6	
	Roots	930	70		
Lewis and Powers (present paper)	Corn	50	6	4	C. p. chemicals and tap water
	Barley	200	8	4	
	Head lettuce	270	3 5	4	C. p. chemicals and distilled water

[1] Fresh-weight basis

[2] See footnote 4

Iodine analyses were made of certain plant materials grown in the above-described experiments. In general, the iodine content of the plant material was greatly increased in the presence of added iodine, the iodine content being more or less linearly related to the concentration of added iodine By assuming that plant material grown in the complete absence of iodine in the solutions would be essentially iodinefree, and assuming further that the linear relation of iodine content to iodine concentration holds at low iodine concentrations, it is possible to estimate the iodine concentration in the control solution from the iodine content of plant materials grown in the control solution and of materials grown under otherwise identical conditions in the presence of known concentrations of iodine. The data on which three sets of such calculations are based are presented in table 2. The apparent iodine content of the control solutions, as well as the results of similar calculations for the control solutions of other investigators, are shown in table 3. The apparent iodine content of the writers' control solutions in most cases is of the order of 1 p. p. b. The unpurified solutions show lower iodine concentrations than the control solutions used by the other investigators cited.

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[6] The standard error of the mean is used in this paper.

Two possible sources of error may affect the above calculations. In the first place, the linear relation between iodine concentration in the solution and iodine content of the plant material may not hold at low iodine concentrations. In general, the error would be expected to be greater, the greater the difference between the iodine concentrations in the known and the unknown solutions. That this source of error is not serious in the writers' calculations is indicated by the fact that such calculations based on three plant species grown in known iodine concentrations varying over wide ranges are in substantial agreement.

In the second place, while practically all of the iodine in the high iodine-content plant material must have come from the solution, this is not necessarily true of the iodine in the low iodine-content plant material. A substantial fraction of the iodine in such material may have arisen from atmospheric or other sources of contamination. This consideration would tend to make the calculated iodine content of the solution a maximal value.

PLANT EXPERIMENTS WITH PURIFIED SOLUTIONS

Two experiments were conducted with tomatoes (*Lycopersicum esculentum*) grown in solutions prepared from purified chemicals and redistilled water. Glass culture vessels were thoroughly cleaned with acids, and special precautions were taken against iodine contamination.

In the first experiment, nine 3-liter soft-glass battery jars bearing three plants per jar were used in each series. To facilitate statistical treatment, individual plants were paired on the basis of initial size. A treatment of 0.1 p. p. m. of added iodine was compared with the iodine-free control. After 65 days the plants had attained green weights of about 40 gm. each. No yield response to iodine treatment was obtained, the mean difference between paired plants in favor of the iodine treatment being 0.85+1.5 gm. At this time two plants per jar were harvested, leaving the best paired plant in each jar. These plants were permitted to grow to maturity (104 days). Neither fruit, tops, nor roots showed any response to iodine treatment.

A second experiment was carried out in 16 shallow pyrex baking dishes with glass lids drilled to accommodate the stems of the plants. Six seedlings per dish were started. After 30 days the plants had green weights of 6 to 7 gm. each. The mean of the differences between mean weights of plants in paired jars was 0.25±0.10 gm. in favor of the plants grown in the presence of 0.1 p. p. m. of added iodine. At this time the plants were thinned to two per jar and permitted to grow to the blooming stage. There was no response of either tops or roots to iodine treatments after 82 days of growth.

Sugar beets (*Beta vulgaris*) were grown for a short period in purified nutrient solutions in soft-glass battery jars. After 3 weeks of growth, there were no significant differences between the mean weights for the various treatments, which varied from 4.40 to 4.65 gm. Stoklasa (*22*) obtained a significant response to iodine treatment at 30 days after germination.

Iodine analyses were made of various parts of tomato plants grown in the presence and in the absence of added iodine. These analyses are presented in table 4. They indicate that under the conditions of these experiments the distribution of iodine in the tomato plant was fairly uniform.

TABLE 4.—*The iodine content of tomato plants grown in "iodine-free" solutions and in the presence of added iodine*

Iodine added	First experiment, iodine content of					Second experiment iodine content of 82-day-old whole plants
	65-day-old whole plants	Parts of 104-day-old plants				
		Leaves	Stems	Roots	Fruit	
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
Iodine-free solutions	[1]0.29	0.10	0.10	[1]0.10	[1]0.10	[1]0.11
0.1 p. p. m.	.34	.21	.19	.24	.15	3.6
0.5 p. p. m.						10.2

[1] Duplicate analyses were not made because of insufficient plant material.

The iodine content of the plant materials from the iodine-free cultures were found to approximate 100 p. p. b., and in one case with younger plants to be 290 p. p. b. The origin of this relatively high iodine content is a question of considerable importance. A calculation based on the volume of nutrient solution used to produce 1 gm. of dry plant material indicates that a residual iodine concentration of 0.001 p. p. b. in the iodine-free solutions could have contributed only approximately 0.5 p. p. b. of iodine to the plant material.

The small size and low iodine content of seeds in general seems to preclude a significant iodine contribution from this source. For example, a tomato plant weighing 10 gm. would receive an iodine contribution of only 0.01 p. p. b. from a seed weighing 1 mg. and having an iodine content of 100 p. p. b.

Two iodine analyses of the greenhouse air made during these investigations gave an iodine content of 0.01 and 0.04 γ [7] per cubic meter of air. Each sample was collected over a period of 1 to 3 months and therefore represents a mean air iodine content. The lowest air iodine content among those summarized by McClendon (*15*) is 0.04 γ per cubic meter.

After the completion of the plant experiments, large sheets of filter paper were suspended in the greenhouse and kept there for 72 days. Although the amount of accumulated dust was small, the iodine content of the paper increased 0.5 γ per square foot. This increase is equivalent to approximately 60 p. p. b. of iodine. It will be noted that the latter figure is similar in order of magnitude to the iodine content of the plants grown in the iodine-free solutions. However, the greenhouse air was not especially contaminated with iodine; in fact, it was relatively low in iodine on the basis of our present knowledge of the normal iodine content of the atmosphere. It therefore appears that after the iodine content of the solution is reduced to some point below 1 p. p. b., the iodine content of the air rather than the iodine content of the solution may become a limiting factor in the production of plant materials low in iodine.

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[7] The microgram (γ) is 0.001 mg.

EXPERIMENTS WITH MICRO-ORGANISMS

An initial experiment was conducted with representative bacterial species to find whether any of these species had iodine requirements large enough to be detected by a simple technique. Species of *Bacillus*, *Pseudomonas*, *Escherichia*, *Phytomonas*, and *Aerobacter* on repeated subculturing in iodine-free inverted-sucrose medium failed to exhibit any diminution of growth. Treatments with added iodine at rates of 0.01, 0.1, and 1 p. p. m. were tried as well as the iodine-free control. Subculturing was always from the iodine-free control culture. Although the calculated original iodine content of the medium amounted to only a few parts per billion, atmospheric contamination may have raised the iodine level somewhat since the preliminary trials were carried out in a bacteriological laboratory where the atmospheric iodine might be expected to be relatively high.

More intensive work was carried out on the bacterium *Azotobacter agile*.[8] Aside from the economic importance of the *Azotobacter* and reports of growth responses of *Azotobacter chroococcum* to iodine, the use of this organism permits the use of the more readily purified ethanol as an energy source and the elimination of nitrates from the medium.

This organism has been carried for a year by periodic subculturing in the iodine-free ethanol medium without a progressive reduction in growth rate or an apparent diminution of maximum turbidity of the cultures. The cultures were kept in a room believed to be uncontaminated by iodine. Since the medium is known to have had a maximal iodine concentration of the order of 0.001 p. p. b., it seems likely that iodine is not essential to the growth of *Azotobacter agile* in concentrations greater than 0.001 p. p. b.

TABLE 5.—*Stimulation of nitrogen fixation by Azotobacter agile in the presence of potassium iodide*

Iodine added (p. p. m.)	Total nitrogen fixed per flask after—			
	3 days	5 days	8 days	15 days
	Milligram	*Milligram*	*Milligram*	*Milligram*
0	3.52±0.29	12.8±1.0	25.3±0.9	26.1±0.9
0.01	4.71±.21	16.7±.7	24.7±.6	25.0±.5
0.1	5.33±.22	18.7±.8	27.1±.2	27.3±.2
1	5.70±.11	17.1±.4	26.9±.5	
50	4.94±.40	17.2±.2	25.1±.6	27.0±.4

Under certain conditions, a stimulation of growth of *Azotobacter agile* in the presence of added iodides was observed. In general, growth rate was stimulated while the maximum turbidity was little affected. The results of a typical experiment are presented in table 5. Apparently such results are due to the action of iodine on toxic constituents in the medium rather than to an essential nature of iodine in the nutrition of the organism.

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[8] The original culture of this organism was obtained by Dr. C. P. Hegarty from Dr. W. W. Umbreit of the University of Wisconsin.

Experiments now in progress indicate that iodides antidote the toxic action of copper on *Azotobacter agile*. Thus in the presence of 1 p. p. m. of copper in the substrate, maximum growth was achieved in the presence of 1 and 10 p. p. m. of iodine before any growth was apparent in the controls lacking added iodine, although visible growth was delayed 3 to 4 weeks in both cases. It has not been established, however, that all of the observed responses to iodine additions are due to the antidoting action on copper toxicity, and it is possible that other toxic factors are involved. In this connection, the work of Steinberg (*20*) is pertinent. He found that the toxicity of silver to *Aspergillus niger* is antagonized to a high degree by iodide, less so by bromide, and not at all by chloride. The solubilities of the silver halides appeared to be the important factor.

The growth of a mixed culture of algae in the iodine-free medium was also studied. Additions of iodine between the levels of 1 p. p. b. and 50 p. p. m. produced no apparent effect on growth.

DISCUSSION

The iodine requirements, if any, of most plants must be very low in view of the low normal iodine content of plants. The average iodine content of land plants usually lies in the range of 50 to 200 p. p. b. (*15*). The normal range of boron and manganese content is about 1,000 times this. Since 0.5 p. p. m. of these elements in solution cultures is sufficient for normal plant growth, it appears likely from analogy that an iodine concentration of the order of 1 p. p. b. would be adequate. Since nutrient solutions prepared from ordinary c. p. chemicals apparently have iodine concentrations of this order, the need for nutrient solution purification is obvious.

The presence of an iodine content in the normal range in the tomatoes grown in the specially prepared iodine-free solutions makes it impossible to state definitely on the basis of these experiments that iodine is not essential to the tomato. To show irrefutably that iodine is nonessential, plant material substantially lacking iodine must be grown.

It is possible that the iodine found in these plant tissues was derived from the atmosphere. In spite of the very low iodine content of the air, the observed accumulation of iodine would be no more striking than that of other substances. For example, if plants were able to absorb iodine from air containing 0.02 γ per cubic meter in the same relative proportion as they do carbon dioxide, this could account for an iodine contribution of the order of 100 p. p. b. More rigorous proof of the nonessential nature of iodine would therefore seem to require the use of air purification and unusual precautions against incidental contamination.

The distribution of iodine throughout the plant parts was found to be fairly uniform at both of the iodine levels investigated. Perusal of the literature reveals that, in general, fruits, starchy parts, roots, and seeds are lowest in iodine content, while green parts, especially leaves, are richest. It has frequently been suggested that iodine follows the transpirational stream in plant tissues.

The evidence seems clear that iodine is not essential to *Azotobacter agile*, although stimulations from added iodine may occur as a result of other effects on the environment. It seems possible that a similar relation may be found to hold for plants. The erratic nature and relatively small magnitude of the growth responses of plants to iodine, as well as the lack of specific deficiency symptoms, supports the view that iodine acts by ameliorating detrimental aspects of the environment. Here also should be mentioned the lack of evidence of widespread crop failure in extremely goiterous regions.

EFFECTS OF IODINE ADDITION TO SOILS

EXPERIMENTAL RESULTS

In accord with the second line of attack mentioned in the introduction, preliminary field-plot and greenhouse trials with iodine were conducted on a number of Oregon soils with various crops. Potassium iodide additions equivalent to 2 to 6 pounds of iodine per acre were applied following a complete fertilizer treatment. The iodine was applied in the form of a dilute solution before seeding in the case of annual plants, and during the early seasonal growth stages in the case of perennials.

While the results from some 40 such trials were largely negative, 2 soils were subjected to statistically interpretable tests. Replicated pot-culture trials were conducted with alfalfa (*Medicago sativa*) and red clover (*Trifolium pratense*) on Aiken clay loam and Deschutes sandy loam. The Aiken soil had given indications of a response of alfalfa to iodine treatment.[9] The Deschutes soil came from Deschutes County, Oreg., where endemic goiter in human beings and farm animals has long been recognized. The soil itself was found to contain the low iodine content of 0.5 p. p. m.

The data for the first three cuttings on each trial are given in table 6. The yield data for further cuttings were erratic. Five and ten jars, respectively, were used per treatment in the alfalfa and red clover trials. Each soil received a complete fertilizer treatment before seeding.

Yield increases that seem to be statistically significant were obtained with the Aiken soil only, on the first and second cuttings. A slight response was found with the first cutting only on the Deschutes soil. It may be noted that for the first cuttings of red clover on both soils, and for the first cutting of alfalfa on the Aiken soil, the yield responses are proportionately greater when the green weights are considered; that is, "succulence" as well as total yield has been increased.

The crude protein content as well as the yield was increased in those cases in which iodine responses were obtained. This is of interest in view of Brenchley's (*3*) observation that nontoxic iodine dosages frequently increased the nitrogen content of barley and legumes.

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[9] CARLSON, W. E. A CONTRIBUTION TO THE ROLE OF IODINE IN THE NUTRITION OF CERTAIN PLANTS. 83 pp. Corvallis, Oreg. (Thesis, Oreg. State Col.) Carlson found the closely related Olympic series to be the only one of four Oregon soils investigated that responded to potassium iodide additions in preliminary trials. The Aiken soil was taken from the same hillside as Carlson's Olympic soil. The samples were taken from a virgin profile where contamination with spray residues was out of the question.

TABLE 6.—*The effects of potassium iodide treatment on yields of alfalfa and red clover grown on Aiken clay loam and Deschutes sandy loam, 1937–38*

Item		Check	2 pounds iodine per acre	4 pounds iodine per acre
Aiken clay loam				
Alfalfa, seeded June 1937, mean yields				
First cutting, Sept. 27, 1937				
Green weight	grams	7.60±0.45	10.40±0.45	10.80±0.45
Dry weight	do	2.85±0.22	3.65±0.30	3.75±0.15
Second cutting, Dec. 2, 1937				
Dry weight	do	5.90±0.07	6.40±0.30	7.50±0.67
Third cutting, Mar. 17, 1938				
Green weight	do	33.0±2.2	40.0±3.8	33.0±1.5
Dry weight	do	6.80±0.40	8.20±1.50	6.50±0.30
Iodine content (combined first and second cuttings (dry weight basis))	parts per billion	384	415	361
Red clover, seeded October 1937, mean yields				
First cutting, May 26, 1938				
Green weight	grams	209±18	262±11	255±8
Dry weight	do	51.0±5.0	61.0±2.6	57.1±2.7
Second cutting[1]		(1)	(1)	(1)
Third cutting, Aug. 24, 1938				
Green weight	do	112±4	105±3	113±3
Dry weight	do	29.3±1.3	28.3±1.0	29.6±2.1
Iodine content				
First cutting	parts per billion	376	213	279
Third cutting	do	276	198	198
Protein content				
First cutting	percent	15.55	16.89	17.63
Third cutting	do	15.24	11.85	14.85
Deschutes sandy loam				
Alfalfa, seeded June 1937, mean yields				
First cutting, Sept. 27, 1937				
Green weight	grams	5.60±0.38	6.00±0.22	6.20±0.60
Dry weight	do	1.95±0.15	2.10±0.22	2.10±0.22
Second cutting, Dec. 2, 1937				
Dry weight	do	5.85±0.60	5.15±0.45	5.95±0.38
Third cutting, Mar. 17, 1938				
Green weight	do	40.00±1.5	34.0±3.8	32.0±3.8
Dry weight	do	8.10±0.30	7.4±0.67	6.90±0.60
Iodine content (combined first and second cuttings (dry-weight basis))	parts per billion	26	100	415
Red clover, seeded October 1937, mean yields				
First cutting, May 26, 1938				
Green weight	grams	191±10	21 ±7	206±5
Dry weight	do	46.6±3.3	17.7±1.0	45.9±1.1
Second cutting, July 15, 1938				
Green weight	do	140±10	116±11	120±9
Dry weight	do	31.7±4.1	26.2±2.8	28.7±2.2
Third cutting, Aug. 24, 1938				
Green weight	do	45.4±4.9	40.0±5.6	42.6±2.9
Dry weight	do	11.0±1.1	9.1±1.2	10.3±0.6
Iodine content				
First cutting	parts per billion	186	492	712
Second cutting	do	277		612
Third cutting	do	132		486
Fourth cutting	do	189		484
Protein content				
First cutting	percent	16.94	17.5	17.54
Second cutting	do	14.91	16.10	15.38

[1] Lost

The plant iodine content was related to the iodine treatment in the case of the Deschutes soil which had a basal iodine content of 0.5 p. p. m., but not in the case of the Aiken soil which had the relatively high iodine content of 16.4 p. p. m. The iodine in the latter soil was largely present in the organic matter, as was shown by extraction with solvents that varied in their ability to extract humus. Thus, a water extraction yielded 1.4 p. p. m. or 7 percent of the total iodine in the soil; a 2-percent potassium carbonate extraction yielded 7.7 p. p. m. or 47 percent; while a 1-percent potassium hydroxide extraction yielded 12.3 p. p. m. or 75 percent. The water extract was almost colorless, the potassium carbonate extract was cherry red, while the potassium hydroxide extract was very dark in color.

The difference in the ability of these two soils to retain iodine was also shown by the following leaching experiments. Small samples of soil which had been treated with 1 and 10 p. p. m. of added iodine were placed in duplicate in small Büchner funnels and leached for 2 months with small daily additions of distilled water. The recovery of total iodine from the Deschutes sandy loam was 16 and 46 percent for the soil samples containing 1 and 10 p. p. m., respectively, of added iodine; for the Aiken clay loam the recovery was 0.6, 1.7, and 0.6 percent for the samples containing 0, 1, and 10 p. p. m., respectively, of added iodine.

In accord with the results of other workers, early nontoxic applications of iodine to soils did not give the striking increases in plant iodine content that are noted in solution culture work. The iodine content values obtained are in agreement with those summarized by McClendon (*15*). Incidentally, natural waters were low in iodine, although a few deep wells were found to contain as much as 6 to 120 p. p. b.

DISCUSSION

The results of additions of iodine to Oregon soils have been largely negative. Well-replicated pot-culture trials with legumes on Aiken clay loam have given a small response to iodine treatment that seems to be statistically significant. The few other cases where stimulative actions have been found in preliminary trials must be tested more thoroughly before definite statements can be made.

The largely negative results of iodine fertilization of soils in a region of endemic goiter support the conclusion, based on solution culture trials, that iodine is either not essential in plant nutrition, or is required in very minute amounts only. It is concluded that bona fide responses to iodine treatment are probably due to antagonistic actions in view of the small magnitude and erratic occurrence of soil responses to iodine treatments found by the writers and by other workers.

The stimulation of legume yields on Aiken clay loam is particularly interesting in this connection. The fact that a positive growth response was obtained with low iodine applications on a soil containing a relatively high iodine content, although the iodine content of the plant material was not affected by the iodine treatment; that the response was most apparent on the first cutting and was found only on the first and second cuttings; that the response was greater on the green-weight than on the dry-weight basis; and that the nitrogen content was increased when yield responses were obtained. all point to an indirect effect of the iodine, rather than to the remedying of an iodine deficiency.

The magnitude of the iodine response on the Aiken soil and the evidence for responses on other soils are not great enough to warrant hopes of economic returns from iodine fertilization.

SUMMARY

The effects produced by the addition of small amounts of potassium iodide to nutrient solutions prepared from c. p. chemicals and distilled water have been investigated. Significant increases in yield were not obtained with corn, barley, or lettuce grown in these solutions. The iodine content of the plant material was found to be linearly related to the concentration of added iodine in the solution cultures. On the basis of these data and certain assumptions, the maximal iodine content of the basal nutrient solutions was calculated to be of the order of 1 p. p. b.

Methods were developed for reducing the iodine content of certain nutrient chemicals to less than 1 p. p. b. Using such chemicals and redistilled water, the writers prepared nutrient solutions having an iodine content of the order of 0.001 p. p. b. Tomatoes failed to give growth responses to iodine additions in these solutions.

Azotobacter agile was subcultured at periodic intervals for approximately 1 year in a 1.1 percent ethanol medium having an iodine content of the order of 0.001 p. p. b. without a reduction in the normal growth rate.

Exploratory pot-culture and field-plot trials on a number of Oregon soils with early nontoxic iodine additions have been largely negative. A statistically interpretable greenhouse trial on Aiken clay loam showed small responses with alfalfa and red clover on the initial cuttings. Iodine may act indirectly rather than by remedying a deficiency of an essential element.

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MEETING THE VITAMIN D REQUIREMENT OF PIGS WITH ALFALFA HAY AND WINTER SUNSHINE[1]

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INTRODUCTION

It has been shown by the authors (*3*)[2] that pigs require vitamin D even when receiving rations higher in calcium and phosphorus than are ordinarily recommended. Meeting this requirement is of practical importance and presents a problem under the usual winter-feeding conditions in Minnesota, where rather close confinement of pigs is necessary during the winter months.

It is not generally understood that all common pig feeds, especially grains and protein supplements, are completely lacking in vitamin D. Because of the importance of this vitamin in the nutrition of pigs, further experiments were conducted to determine how it may be provided and also to find the extent of the pig's need for it.

MATERIALS AND METHODS

In addition to feed consumption, weight records, and the gross symptoms of the pigs, the level of calcium in the blood plasma, from single blood samples obtained from tail bleeding, was used to determine the need of the pigs for vitamin D. Since alfalfa hay may contain vitamin D (*6*), and is one of the few roughages used in pig rations, it was fed in each of the three experiments in this investigation. In the first two experiments three lots of hay were used, lot I in the first experiment and lots II and III in the second. At the conclusion of these two experiments the vitamin D content of the three lots of hay was determined. A fourth lot of hay for use in a third experiment was also assayed. This hay was cured by exposure to a number of hours of sunlight approximating that given to hay in the usual manner of curing on farms. For this purpose a small field of alfalfa was mowed at 10:00 a. m. and the hay was put in storage the next day at approximately 1:00 p. m. During the entire curing period the weather was clear, thus permitting the maximum amount of exposure.

Quantities of these four lots of hay were assayed for vitamin D according to the United States Pharmacopoeia XI procedure by which it was possible to determine the approximate number of International Units of vitamin D in each lot of hay. Rachitogenic diet No. 3143 of McCollum et al. (*4*) was employed. The vitamin D content of the four lots of hay, as shown in table 1, was 0.15, 0.39, 1.46, and 0.85 International Units per gram. It should be noted that Wallis (*7*) reports 10.45 International Units for alfalfa leaves and 1.72 for

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[2] Italic numbers in parentheses refer to Literature Cited, p. 648.

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alfalfa stems. The discrepancy between his results and those here shown is apparently due to a difference in healing obtained with the reference oil.

TABLE 1.—*Results of vitamin D assays with rats of the different lots of alfalfa hay fed in the pig experiments*

Material, level of feeding, and quantity consumed during the first 8 days of the assay period	Rats used	Average healing (line test)[1]	Vitamin D per gram (calculated)[2]
	Number		*International Units*
Standard reference oil, 0.4 International Unit daily	10	+2.5	-
Lot I alfalfa, 20 percent of diet, 1.47 gm. average daily intake	12	+1.4	0.15
Lot II alfalfa, 10 percent of diet, 0.69 gm. average daily intake	13	+1.7	.39
Lot III alfalfa, 5 percent of diet, 0.34 gm. average daily intake	9	+3.1	1.46
Lot IV alfalfa, 10 percent of diet, 0.66 gm. average daily intake	15	+3.5	.85

[1] The numerical degrees of healing suggested by Bills et al *(1)* were followed in interpreting the line tests.

[2] The calculated values are not strictly correct since they were made by simple proportion as is customary. It is known that the relationship between the units of vitamin D received and the degree of healing is curvilineal, and that vitamin D units in an unknown may be calculated by arithmetical proportion only when the unknown and the standard reference will give the same degree of healing.

EXPERIMENT 1

PROCEDURE

In experiment 1, begun December 12, 1935, three lots of six pigs each were used. In lot 1 there were one Duroc-Jersey, one Poland China, and four Hampshires. In each of the other two lots there were two Poland Chinas and four Duroc-Jerseys. The pigs were started on experiment shortly after weaning and were fed individually twice daily. All lots in this experiment, as well as in the experiments which follow, were housed in a hog barn and were not allowed access to the outside except as called for by the experimental procedure

The ration fed lot 1 consisted of yellow corn, 86.84 percent; dry-rendered tankage, 11.80 percent; sodium chloride, 0.50 percent; bonemeal, 0.26 percent; limestone, 0.45 percent; and unirradiated yeast, 0.15 percent. This ration provided 0.65 percent of phosphorus and 1.07 percent of calcium. The pigs receiving it were allowed to become rachitic and were then used to test the antirachitic potency of alfalfa. Lot 2 received the above-described ration and had access to the outside at all times. This plan was followed to determine whether pigs so handled would receive enough benefit from winter sunshine to preclude the necessity of adding a vitamin D supplement to their ration. A daily record was kept of the hours of sunshine and the approximate amount of time the pigs spent in their outside lot from the beginning of the experiment until its close. The only change made in the above ration for lot 3 was the substitution of irradiated yeast for unirradiated yeast; thus this ration served as the control since a ration containing 0.15 percent of irradiated yeast had been found by the authors *(3)* to afford complete protection from rickets.

RESULTS

As the body stores of vitamin D became depleted the average percent of calcium in 100 cc. of blood plasma of five of the pigs in lot 1 decreased from 10.47 mg. on January 9 to 6.51 mg. on February 24.

One week later the remaining pig was bled and showed 6.91 mg. All of these pigs showed marked reduction in feed intake and negligible growth or a loss in weight, and two pigs exhibited calcium-deficiency tetany. That these symptoms were the result of a vitamin D deficiency was shown by the fact that the plasma calcium of one of the pigs manifesting tetany increased from 5.58 mg. to 11.05 mg. in 12 days after the pig had received 4 ounces of U. S. P. cod-liver oil. Furthermore, the pigs in lot 3, the control lot, showed no reduction in plasma calcium and their appetites and growth were excellent.

Four of the pigs in lot 1 were given lot I alfalfa as 1 percent of their ration and the remaining pig, one of the two manifesting calcium-deficiency tetany, was given the alfalfa as 5 percent of its ration. However, in order to save the life of this pig 8 ounces of cod-liver oil were administered over a period of 4 days, after which it recovered. After 16 feedings of the ration containing 1 percent of lot I alfalfa, the plasma calcium of each of the pigs was lower than before it was given. Consequently the alfalfa was increased to 5 percent. After 12 feedings of this ration one of the four pigs had a convulsion; it was given 4 ounces of cod-liver oil and removed from the experiment. The remaining three pigs received 16 feedings of this ration but showed no improvement. The alfalfa was then increased to 10 percent of the ration. The lack of recovery from 21 feedings of this ration is not surprising in view of the small amount of vitamin D in this hay (table 1). During this period of the experiment the three pigs, averaging approximately 200 pounds, ate on an average 4.85 pounds of feed per day, and each therefore received but 33 International Units of vitamin D daily.

The pigs in lot 2 received the same ration as those in lot 1, but in spite of having access to an outside lot, they needed more vitamin D during the early part of the experiment than they obtained from exposure to sunlight. This was indicated by the incidence of stiffness and slight reductions of plasma calcium in several of the pigs and by slower average growth for the lot as compared with lot 3, the pigs in the latter lot averaging 7.3 pounds per pig heavier than those in lot 2. This difference, however, developed during the first part of the experiment; during the latter part, February 6 to March 20, lot 2 gained equally as well as lot 3. It is interesting to note that the improvement in growth of the pigs of lot 2 coincided with the period of greater sunshine during which, unless the weather was severe, they spent more time outside.

EXPERIMENT 2

PROCEDURE

In order to make a further study of the value of winter sunshine in curing rickets and of alfalfa hay in preventing rickets two lots of six pigs each were started on experiment December 21, 1936. The pigs, three Duroc-Jerseys and three Poland Chinas in each lot, averaged 45 pounds in weight. The ration fed to lot 1 for the purpose of testing the value of winter sunshine, consisted of corn, 86 percent; dry-rendered tankage, 12 percent; sodium chloride, 0.50 percent; bonemeal, 1.0 percent; and limestone, 0.50 percent. The calcium and phosphorus in the ration were 1.05 percent and 0.63 percent, respectively. Lot 2 received 95 parts of this ration and 5 parts of lot II alfalfa meal.

The inclusion of the alfalfa did not appreciably change the percentages of calcium and phosphorus in this ration as compared with that for lot 1. The ration for each lot was self-fed. Individual weights of the pigs and the feed eaten by each lot were recorded at 2-week intervals.

RESULTS

The data in table 2 show that in 8 weeks there was a reduction in the average plasma calcium values of the pigs in lot 1 from 10.95 mg. to 6.89 mg. Some of these pigs were lame, but lameness was not characteristic of all the pigs in this lot. Since it was clearly evident from the blood picture that these pigs were rachitic, they were exposed to the sunlight for 2 weeks, beginning February 16. The exposure was limited to the period from 10:30 a. m. to 3:30 p. m. Because of cloudy weather they were exposed for an average of only 1.48 hours per day. This treatment caused the plasma calcium to increase to 10.52 mg. (table 2), and this increase was accompanied by a marked improvement in appetite, growth, and appearance. Solar irradiation was continued for another 2 weeks, which resulted in further improvement, although sufficient irradiation may have been given during the first period to account for the continued improvement.

TABLE 2.—*Calcium in 100 cc. of blood plasma of pigs as affected by exposure to sunshine and by dietary supplements during experiment 2, 1936–37*

	Calcium in 100 cc. of plasma in lot 1[1]					Calcium in 100 cc. of plasma in lot 2[2]			
Pig No.[3] and breed	Dec. 21	Feb. 15	Mar. 1	Mar. 15	Pig No.[3] and breed	Dec. 21	Feb. 15	Mar. 1	Mar. 15
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*		*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
1 DJ	10.05	6.96	10.64	11.56	7 DJ	11.01	10.23	11.26	11.26
2 DJ	10.95	6.65	10.69	10.69	8 DJ	10.36	9.31	9.06	11.15
3 DJ	10.69	6.91	9.52	11.56	9 DJ	11.00	8.70	9.82	10.95
4 PC	11.64	7.12	10.49	11.51	10 PC	10.69	10.13	10.54	10.71
5 PC	11.01	7.37	10.74	11.72	11 PC	10.42	9.62	10.03	10.95
6 PC	11.33	6.04	11.05	11.36	12 PC	10.95	10.18	10.74	10.95
Mean	10.95	6.89	10.52	11.40	Mean	10.71	9.70	10.24	11.00

[1] Exposed to sunlight beginning Feb. 16.

[2] Ration included 5 percent of alfalfa meal. For 2 weeks prior to Feb. 15 these pigs received an average of 38 I. U. of vitamin D per animal per day; from Feb. 15 to Mar. 1 they received 183; and from Mar. 1 to Mar. 15, 241.

[3] DJ and PC signify Duroc Jersey and Poland China, respectively.

The blood picture of lot 2 was much more favorable than that of lot 1. From December 21 to February 15 there was an average reduction of only 1.04 mg. in the plasma calcium. Although there was a variation among the pigs in this respect the plasma calcium of each showed some reduction. It was evident that the lot II alfalfa hay furnished enough vitamin D to give some protection from rickets. On February 15 the pigs averaged 102 pounds in weight and for the 2 weeks prior to that date had received an average of 38 International Units of vitamin D per pig per day. On February 15 the supply of this lot of hay was exhausted and another lot (lot III) was fed. That this hay contained enough vitamin D for the pigs was evident from the increase, with the exception of pig 8, in their plasma calcium values in 2 weeks and a further increase for all the pigs in another 2 weeks. From February 15 to March 1 the pigs received 183 International

Units of vitamin D daily per pig, and from March 1 to March 15 they received 241 International Units. In the writers first investigation (*3*) it was found that irradiated yeast as 0.10 percent of the ration failed to prevent rickets but as 0.15 percent it afforded complete protection. Although this yeast was not assayed, according to the manufacturer it probably contained 900 International Units per gram. Thus, approximately 1,700 International Units of vitamin D per pig per day were required. This discrepancy between the amount of vitamin D required by pigs when supplied in irradiated yeast and when supplied in alfalfa hay requires further study.

The difference in the appetite of pigs in lot 1 and lot 2 and the improvement of both in this respect after receiving what was apparently an adequate amount of vitamin D (table 3) illustrates the importance of meeting the need of pigs for this vitamin. This is true even of lot 2 in which only a slight deficiency was encountered as judged by the small reductions of blood-plasma calcium and by the total absence of visible symptoms of rickets. As expected, the increase in feed consumption was accompanied by an improvement in growth. Lot 1 gained 54 percent faster during the 2 weeks after exposure to the winter sunshine, and lot 2 gained 43 percent faster during the 2 weeks after the change from lot II to lot III alfalfa, than during the 2 weeks preceding these changes.

TABLE 3. *Average daily feed consumed per pig at different times during experiment 2 1936–37*

Lot No.[1]	Dec 21–Jan 4	Jan 4–Jan 18	Jan 18–Feb. 1	Feb 1–Feb. 15	Feb 15–Mar 1	Mar 1–Mar 15
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1	2.61	3.05	3.24	3.23	4.48	6.21
2	2.56	3.16	3.72	4.28	5.52	7.29

[1] See footnotes 1 and 2, table 2

EXPERIMENT 3

PROCEDURE

In order to obtain more information on the requirement of the pig for vitamin D, a third experiment was initiated November 15, 1937. Six lots of pigs, averaging 47 pounds in weight, were used for studying (1) the value of winter sunshine and of alfalfa containing 0.85 of an International Unit of vitamin D per gram (lot IV) in curing rickets in pigs, (2) the value of this same alfalfa in preventing rickets, and (3) the effect of the concentration of calcium and phosphorus in the ration on the vitamin D requirement. Each lot consisted of one Duroc-Jersey, two Poland Chinas, and three Hampshires. However, during the course of the experiment one pig was removed from lot 1 and one from lot 3 for failure to eat their food. Records for these pigs have been omitted from the report of this experiment.

The percentages of calcium and of phosphorus in rations of swine vary widely, depending upon the protein supplement used. During summer feeding, when providing vitamin D is not a problem, similar results can be obtained from protein supplements consisting largely of soybean-oil meal or dry-rendered tankage or of meat and bone scraps. For young pigs, when the demand for protein is high, the phosphorus content of such rations is approximately 0.40 percent, 0.60 percent, and 1.2 percent, respectively. Since it was desired to use rations typical of those used in practice, concentrations of phosphorus similar to the above were employed. These rations, shown in table 4, which were self-fed to lots 1, 2, and 3, had a phosphorus content of 0.42 percent, 0.64 percent, and 1.19 percent, respectively. The calcium-phosphorus ratios were 1.5 for rations 1 and 2 and 1.6 for ration 3. Excepting ration 1, the calcium levels were typical of those in swine rations employing the protein supplements mentioned above. Additional calcium was added to ration 1 in order that the calcium-phosphorus ratios in the three rations might be similar.

TABLE 4.—*Rations used in experiment 3, 1937–38*

Ration 1		Ration 2		Ration 3	
Component	Proportion	Component	Proportion	Component	Proportion
	Percent		*Percent*		*Percent*
Yellow corn	76.66	Yellow corn	75.13	Yellow corn	70.50
Soybean oil meal (80 percent), tankage (20 percent)	22.00	Soybean oil meal (80 percent), tankage (20 percent)	22.30	Soybean oil meal (80 percent), tankage (20 percent)	23.25
Sodium chloride	.50	Sodium chloride	.50	Sodium chloride	.50
Limestone	.84	Limestone	.17	Limestone	.25
		Bonemeal	1.60	Bonemeal	5.50

Pigs of lots 4 and 5 received the same rations as lots 1 and 2 except that 5 percent of the ration consisted of lot IV alfalfa hay containing 0.85 of an International Unit of vitamin D per gram. This hay was cured under ideal conditions. The pigs of lots 4 and 5 were confined indoors. It should be recalled that in experiment 1 pigs fed a ration containing no vitamin D, but given voluntary access to an outside runway, did not receive sufficient exposure to sunlight during December and January; that in experiment 2 rickets was cured when pigs were given exposure to winter sunshine; and that lot III alfalfa, as 5 percent of the ration, contained enough vitamin D (1.46 International Units per gram) to meet the pigs' requirement for this vitamin. It was, therefore, considered desirable to feed the pigs of lot 6, in addition to ration 1, lot IV alfalfa hay, and also to allow them voluntary access to an outside runway. Since alfalfa hay is commonly self-fed to hogs on farms, this method of feeding the hay was followed with this lot. Feed and weight records for the three lots were taken at biweekly intervals.

RESULTS

The calcium analyses of the blood plasma and the feed intake of the pigs in experiment 3 are shown in tables 5 and 6, respectively. As was expected, the calcium content of the blood plasma of representative pigs was normal 1 week after the beginning of the experiment. After 8 weeks, on January 11, all of the pigs in lot 1 except No. 1 and all in lot 2 except No. 7 showed plasma calcium concentration considerably below normal. The two exceptions, litter mates, were removed from their respective lots on January 13 and given ration 1, the low-calcium, low-phosphorus ration which pig No. 1 had been receiving. Not until February 7, 12 weeks after the start of the experiment, was there a reduction in the plasma calcium of these two pigs; namely, to 9.65 mg. for No. 1 and 9.14 mg. for No. 7. These pigs, subjected to the same preexperimental treatment as the others, presented such a marked contrast to the other 9 pigs in lots 1 and 2 that there would appear to be important hereditary differences among pigs in their requirement for vitamin D.

One pig in lot 3, No. 18, receiving ration 3, and one in lot 4, No. 22, receiving ration 1 supplemented with lot IV alfalfa, also showed slight reductions in plasma calcium values at the end of 8 weeks. However, when calcium analyses were next made No. 22 had a plasma calcium value of 10.33 mg. on February 4 and No. 18 one of 11.16 mg. on February 7.

It is interesting to note that the higher calcium and phosphorus rations fed to lots 2 and 3 as compared with lot 1 apparently reduced the rate of utilization of the vitamin D stores for the 8 weeks, because the average plasma calcium value for lot 2 was 1.5 mg. higher than for lot 1, and none of the pigs in lot 3 showed an appreciable reduction in this value except No. 18, as has been mentioned.

TABLE 5.—*Calcium in 100 cc. of blood plasma of pigs as affected by exposure to sunshine and by dietary supplements during experiment 3, 1937–38*

Lot No.	Pig No. and breed[1]	Calcium in 100 cc. of plasma on—							
		Nov. 23	Jan. 11	Jan. 26	Feb. 1	Feb. 7	Feb. 8	Feb. 21	Feb. 28
		Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
1[2]	1 DJ	10.31	10.57			9.65		8.91	
	2 PC	10.25	6.65	6.18			5.97		
	3 PC		6.70	7.90			7.73	5.87	
	5 H		5.76	6.12			7.27		
	6 H	11.39	7.91	8.20			8.72	8.21	
2	7 DJ	10.31	11.26			9.14		7.23	
	8 PC	10.79	7.54	4.57					
	9 PC		9.33	11.21					
	10 H		8.10	10.69					
	11-H	11.06	9.87	12.27					
	12 H	6.40	11.26						
3	13 DJ	11.33	10.77			11.26			10.14
	14 PC	11.50	12.11			11.37			10.29
	16 H		11.36			12.20			9.15
	17 H	11.17	11.76			11.11			10.14
	18 H		9.53			11.16			9.72
4[3]	19 DJ	10.52	10.82		11.21				
	20 PC	11.12	11.01		11.32				
	21 PC		11.91		12.20				
	22 H		9.92		10.33				
	23 H	10.74	10.97		11.11				
	24 H		10.72		10.69				
5[3]	25 DJ	10.63	11.31		11.68				
	26 PC		10.72		11.06				
	27 PC	10.90	10.57		11.16				
	28 H		10.62		11.21				
	29-H	11.50	11.41		11.26				
	30-H		11.11		11.16				
6[4]	31 DJ	10.01	10.92		10.23				
	32 PC		11.71		11.21				
	33-PC	10.52	10.42		11.16				
	34-H		10.97		11.11				
	35 H	11.77	10.87		10.48				
	36 H		11.26		11.83				

[1] DJ, PC, and H signify Duroc Jersey, Poland China, and Hampshire, respectively.

[2] Received basic diet only, but lots 2 and 3 received more calcium and phosphorus than lot 1. The pigs in lot 2 were exposed to sunlight, beginning Jan. 13.

[3] Ration included 5 percent of alfalfa hay; pigs confined indoors.

[4] Ration included 5 percent of alfalfa hay, pigs had access to an outside runway.

TABLE 6.—*Average daily feed consumed per pig at different times during experiment 3, 1937-38*

Lot No.[1]	Nov. 16–Nov. 30	Nov. 30–Dec. 14	Dec. 14–Dec. 28	Dec. 28–Jan. 11	Jan. 11–Jan. 25	Jan. 25–Feb. 8	Feb. 8–Feb. 22	Feb. 22–Mar. 1
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1[2]	3.08	3.13	3.79	4.02	3.41	4.72		
2[3]	2.71	3.12	3.70	4.15	3.64	4.77		
3	2.73	2.68	2.81	3.56	4.27	5.98	5.03	5.97
4	2.65	3.19	3.45	4.21	4.40	4.12		
	2.80	2.96	2.91	4.02	3.95	3.69		
	2.87	3.34	3.62	4.37	4.57	4.03		

[1] See Footnotes 1, 3, and 4, table 5.

[2] Lot 1 continued until Mar. 1, but feed records are not given for additional periods because of changes in ration.

[3] Lot 2 stopped Feb. 1.

Since pigs 2, 3, 5, and 6 on ration 1 were rachitic, it was decided to feed them the alfalfa that lots 4, 5, and 6 had been receiving. Accordingly, the hay was self-fed beginning January 13, and a record was kept of its consumption. By January 28 there was a slight increase in the plasma calcium of three of these pigs and a reduction in that of the fourth. By February 8, 26 days after the feeding of the hay had started, during which time they ate a quantity of hay consisting of 6.9 percent of their ration, the plasma calcium values of Nos. 5 and 6 were still higher, indicating some benefit from the vitamin D in the hay. However, by February 21 the calcium values for these pigs were again lower. Pigs 3 and 6 were continued on this ration until the close of the experiment on March 1 without any noticeable improvement in the rachitic condition.

The fact that lot 3 receiving ration 3, high in calcium and phosphorus, showed no need for vitamin D at the time the attempt to bring about recovery in lot 1 by means of alfalfa hay was meeting with failure, seemed to indicate that pigs require little if any vitamin D when receiving sufficiently high concentrations of calcium and phosphorus in the rations, and that, therefore, it should be possible to cure rickets by feeding such a ration. Accordingly, on February 8, pigs 2 and 5 were given ration 3. This did not improve their condition, which instead, became progressively worse; No. 2 had to be removed from the experiment 1 week later and by March 1 No. 5 had lost 6 pounds. Since pigs 1 and 7 had become slightly rachitic, as shown by reductions of blood-plasma calcium to 9.65 mg. and 9.14 mg., respectively, they too were changed to the high mineral ration on February 8, but the change was without benefit as shown by further reductions of blood-plasma calcium on February 21 to 8.94 mg. and 7.23 mg., respectively. Pigs 3 and 6 were changed to this ration on February 21 and continued on it until the close of the experiment on March 1, but the change was likewise without benefit.

In order to test the value of winter sunshine for curing rickets, the five pigs in lot 2 were given solar irradiation, beginning on January 13. For 14 days they received, on an average, approximately 45 minutes of irradiation per day. The remarkable benefit of this treatment is shown by the increase to normal in plasma calcium values on January 28. The stiffness exhibited by several of the pigs also disappeared.

The use of the high calcium and phosphorus ration 3 for lot 3 was distinctly beneficial in reducing the rate of the utilization of the body stores of vitamin D. It was not until February 28 that there was any evidence from the plasma calcium values of a need for this vitamin. At that time there was a reduction of plasma calcium in each pig and an average reduction of 1.53 mg. for the lot as compared with the values on February 7. It is believed that if these pigs had been continued on experiment they would eventually have become as rachitic as the pigs in lots 2 and 3. From the standpoint of practical feeding it would be more costly to provide rations as high in calcium and phosphorus as ration 3 in order to obviate the need for added vitamin D during the months of little sunshine and confinement than to use a vitamin D supplement. This is suggested by the finding that the vitamin D requirement of pigs can be met by forced exposure to winter sunshine and by the results with lots 4, 5, and 6, to be discussed later, and also by the observation that lot 3 tried to avoid eating the bonemeal in the ration by allowing it to settle to the bottom of the feeder. Bohstedt (*2*) has also found that rations unusually high in minerals are not conducive to the best results with pigs.

It may be seen from the data in table 5 that lots 4 and 5, which received the same rations as lots 1 and 2 except that 5 percent of alfalfa containing 0.85 of an International Unit of vitamin D per gram was included, still showed normal plasma calcium values on February 4, 3½ weeks after lots 1 and 2 were severely rachitic. The pigs in lot 6 also showed normal plasma calcium values at that time. These results could be expected in view of the fact that the average hay consumption of these pigs was 5.05 percent of their ration and from the further fact that they spent sufficient time in their outside runway to receive some exposure to sunshine. In order to facilitate the more extensive studies with lots 1 and 2 the last three lots of the experiment were discontinued on February 5.

It is apparent from this experiment that pigs require more vitamin D to recover from severe rickets than is required to protect them from the disease, even when their body stores of the vitamin are partially depleted. It is, therefore, particularly noteworthy that there was such a rapid recovery of the pigs in lot 2 as a result of exposure to January sunshine. This finding, agreeing with that of Sloan (*5*) for poultry in the vicinity of Ithaca, N. Y., is of practical importance in winter swine feeding, particularly in view of the generally accepted belief that winter sunshine is of no value in generating vitamin D in livestock.

SUMMARY AND CONCLUSION

The growing pig's requirement for vitamin D was investigated with fall-farrowed pigs of the Duroc-Jersey, Hampshire, and Poland China breeds in 3 different years. In some of the tests the pigs were allowed to become rachitic, as judged by reductions in blood-plasma calcium, and were then exposed to winter sunshine or were given alfalfa hay. In other tests attempts to provide the needed vitamin D were made at the beginning of the experimental period by confining the pigs indoors and feeding alfalfa as the source of the vitamin or by allowing them voluntary access to an outside runway with or without alfalfa hay. Four lots of alfalfa hay, all of which were assayed for vitamin D by the U. S. P. XI procedure, were used. The effect of low, medium, and high concentrations of calcium and of phosphorus on the vitamin D requirement was also studied.

It was found that rickets was cured in pigs exposed to January sunshine for an average of 45 minutes per pig per day for 2 weeks; no tests with shorter exposures were made. Similar results were obtained in another experiment with pigs exposed to February sunshine. Pigs fed indoors and allowed voluntary access to an outside runway did not receive sufficient solar irradiation during December and January. When alfalfa, containing 0.39 of an International Unit of vitamin D per gram was fed as 5 percent of the ration the pigs became rachitic. This condition was cured by using another alfalfa hay containing 1.46 International Units per gram as 5 percent of the ration. This hay provided approximately 200 to 250 International Units of vitamin D daily per pig. Pigs receiving an alfalfa hay containing 0.85 of an International Unit per gram, as 5 percent of the ration. did not become rachitic, although this hay failed to cure rickets at this percentage of the ration. Pigs allowed access to an outside runway and self-fed this alfalfa ate 5.05 percent of their ration as hay and did not become rachitic. This lot of hay was cured under ideal conditions, having been mowed in the forenoon and put in storage the afternoon of the next day during which time clear weather prevailed. The need for vitamin D was found to be inversely proportional to the calcium and phosphorus content of the ration, at the levels fed in these experiments.

The effectiveness of meeting the pig's requirement for vitamin D by feeding alfalfa hay and by allowing access to winter sunshine would depend, of course, upon the extent to which the hay had been exposed to sunshine during the curing process and upon the amount of sunshine and the severity of the weather during the winter months.

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MEIOTIC BEHAVIOR OF PHLEUM PRATENSE, PHLEUM SUBULATUM, AND THEIR F_1 HYBRID [1]

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INTRODUCTION

The somatic chromosome number 42 has been reported for the American or "cultivated" timothy (*Phleum pratense* L.) by Gregor and Sansome (*4*),[2] by Avdulow (according to Müntzing (*11*)), by Müntzing (*11*), and by Nordenskiöld (*18*). Since 14- and 28- chromosome species of *Phleum* are known, Müntzing (*11*) concluded that the basic number of the genus is 7 and that *P. pratense* is, consequently, a hexaploid. Gregor and Sansome (*4*) and Gregor (*3*) obtained 4 hexaploid plants from seed produced on triploid F_1 plants of the hybrid *Phleum pratense* ($2n=14$) × *P. alpinum* ($2n=28$). One of these plants was cross-fertile with hexaploid *P. pratense*. On the basis of these results and the chromosome behavior in some plants that he considered to be derivatives of the hybrid *P. pratense* ($2n=42$) × *P. pratense* ($2n=14$), Müntzing (*11*) concluded that hexaploid *P. pratense* had the genomatic constitution *NNAABB*, where *N*, *A*, and *B* were different genomes of 7 chromosomes. This conclusion is further supported by Müntzing's (*11*) observation of 21 bivalents at metaphase I in *P. pratense* and by Nordenskiöld's (*18*) statement that multivalent associations were not found in this species. In a later paper, Müntzing and Prakken (*13*) did not exclude the possibility of quadrivalent formation but stated that such association was very rare. On the other hand, Nordenskiöld (*18*) reported that 14 bivalents occurred at metaphase I in hybrids of *P. pratense* ($2n=42$) × *P. pratense* ($2n=14$), indicating at least partial homology between the chromosomes of 2 of the genomes of hexaploid timothy. This conclusion was supported by the meiotic behavior in 63-chromosome plants of *P. pratense* that Müntzing and Prakken (*13*) obtained from twin seedlings. These plants had each genome represented 3 times and, as a result, might have been expected to show a considerable frequency of trivalents and a total of 21 trivalents and bivalents. Instead, the authors reported that trivalents were rare and that the sum of the trivalents and bivalents was seldom less than 28. On the basis of these results, they concluded that the genomatic constitution of *P. pratense* is $NNA_1A_1A_2A_2$, where A_1 and A_2 are sufficiently homologous to permit formation of bivalents between chromosomes of the 2 genomes.

The object of the investigation reported in this paper was to obtain further information relative to the genomatic constitution of hexaploid *Phleum pratense* and the interspecific relationships in the genus *Phleum*. The work was done at the United States Regional Pasture Research Laboratory,[3] State College, Pa.

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[1] Received for publication January 27, 1941.

[2] Italic numbers in parentheses refer to Literature Cited, p. 658.

[3] Maintained in cooperation with the agricultural experiment stations of the 12 Northeastern States.

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MATERIAL AND METHODS

Panicles of a male sterile plant of *Phleum pratense* were dusted with pollen from a plant of *P. subulatum* (Savi) Aschers. and Graebn., and three seeds were obtained. All the seeds germinated, and two plants grew to maturity. The third plant was lost, owing to an accident of greenhouse culture rather than to any inherent weakness. Plants from the clone of the *P. pratense* parent and from seed produced under bag on the *P. subulatum* parent were grown in the greenhouse at the same time as the hybrid plants for use in comparative studies.

Chromosome numbers of the parents and F_1 were determined from paraffin sections of root tips prepared in the same manner as described previously for *Lolium perenne* L. (*14*). For meiotic studies, fresh acetocarmine smear slides were prepared from material fixed in acetic alcohol.

EXPERIMENTAL RESULTS

MEIOSIS IN PHLEUM SUBULATUM

Determinations from root-tip preparations and from microsporocytes showed that *Phleum subulatum* was diploid ($2n = 14$). Thirty-six sporocytes at diakinesis and 149 sporocytes at metaphase I were examined, and, of these, 183 (98.9) percent had 7 bivalents. In each of 2 sporocytes (1.1 percent of those examined) 2 univalents and 6 bivalents were found. The total chiasma frequency in 20 sporocytes at metaphase I ranged from 10 to 15, with an average of 12.95 per sporocyte, or 1.85 per bivalent. The average frequency of terminal chiasmata was 12.5, giving a terminalization coefficient of 0.96. As indicated from these data, the majority of bivalents had 2 chiasmata, 1 in each arm, resulting in ring-shaped bivalents. However, on an average, 1.35 bivalents per sporocyte were of the open type, i.e., with a single chiasma. A sporocyte at metaphase I with 7 ring-shaped bivalents is shown in figure 1, *B*, while the sporocyte in figure 1, *C*, has 2 open bivalents. Figure 1, *A*, shows a sporocyte at diakinesis with 7 ring-shaped bivalents. In this sporocyte 1 bivalent lies on the nucleolus, while the remaining 6 are free from it. Since this was a regular occurrence at diakinesis, it is probable that only 1 member of the haploid complement bears a nucleolus organizer, although this conclusion could not be verified by studies of midprophase.

Of 122 sporocytes examined at anaphase I, none showed lagging univalents, the distribution in all cases being 7 chromosomes to each pole. Likewise, no evidence of chromosome loss was found at interphase I. At anaphase II some chromosomes lagged; these were left in the cytoplasm in one or more cells of 2.47 percent of the 202 quartets observed. The origin of the lagging univalents at anaphase II was not determined. In other species such laggards frequently are daughter univalents from chromosomes that had lagged and divided equationally at anaphase I. Although no laggards were observed at anaphase I in this material, it is possible that examination of a larger number of sporocytes would have revealed some lagging univalents.

MEIOSIS IN PHLEUM PRATENSE

Attempts to study midprophase in *Phleum pratense* were only partially successful. Pairing appeared to be predominantly as bivalents, although occasional exchanges of partners were observed. It

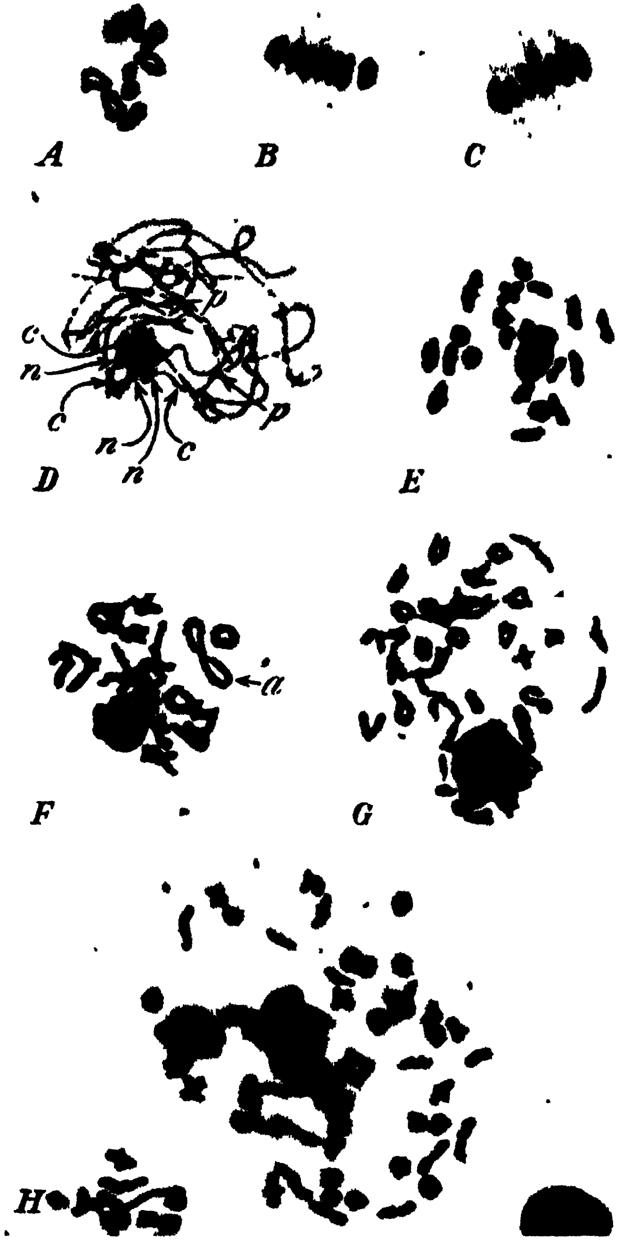

FIGURE 1. *A*, Diakinesis in *Phleum subulatum*; *B*, metaphase I in *P. subulatum* with 7 ring-shaped bivalents, *C*, metaphase I in *P. subulatum* with 2 open bivalents; *D*, midprophase in *P. pratense*, the arrows at *n* indicating nucleolus organizers; *c*, centromere; and *p*, pycnotic knobs; *E*, diakinesis in *P. pratense* with 21 bivalents; *F*, diakinesis in *P. pratense* showing quadrivalent at *a*; *G*, diakinesis in sporocyte of *P. pratense* with 84 chromosomes; *H*, diakinesis in sporocyte of *P. pratense* with 126 chromosomes. × 850.

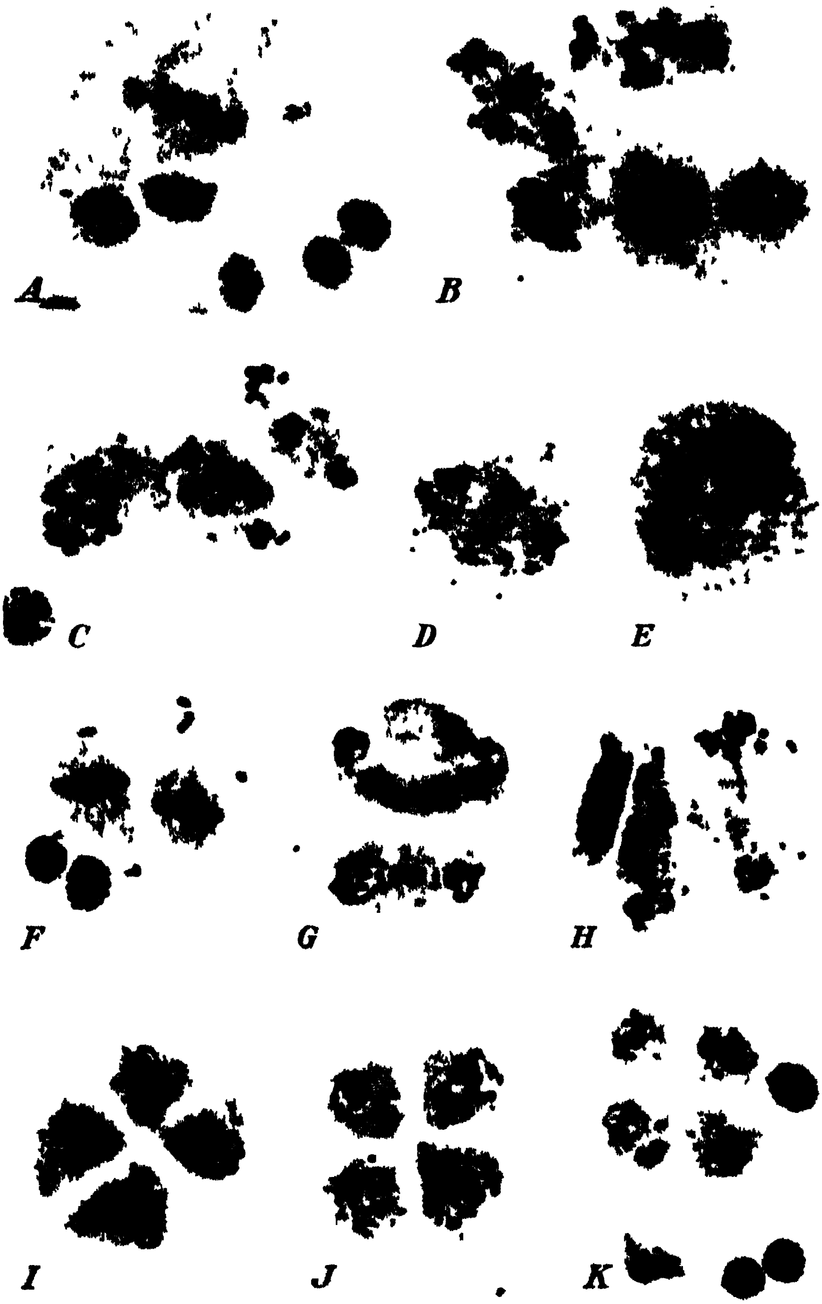

FIGURE 2.—Meiosis in *Phleum pratense* × *P. subulatum*. *A*, Metaphase I, *B*, adjacent metaphase I and anaphase I sporocytes, *C*, adjacent anaphase I and anaphase II sporocytes, *D*, anaphase I with dicentric bridge, *E*, telophase I with dicentric bridge and acentric fragment, *F*, metaphase II, *G*, telophase II, *H*, *I*, *J*, *K*, quartets. × 850.

The data on their frequency were too limited to permit a determination of what proportion of the daughter univalents lagged at anaphase II.

Quartet formation was so irregular that statistical analysis was not possible. Chromatin clumps or micronuclei were found in all quartets (fig. 2, *G* to *K*). Some of these chromatin clumps appeared to be single daughter univalents, while others apparently consisted of two or more univalents that had clumped together. Sporocytes in which the univalents formed a micronucleus midway between the quartet nuclei (fig. 2, *G*) were common. Frequently this chromatin clump had been cut in two by the cell wall formation (fig. 2, *I*), but in other sporocytes wall formation was inhibited, resulting in two or three cells instead of the normal four. In rare instances one cell of the quartet had a normal-appearing nucleus not accompanied by micronuclei or chromatin clumps (fig. 2, *K*).

MORPHOLOGY AND FERTILITY

The parental species used in this study have been described by Hitchcock (*5*). Except in respect to the following three characteristics, the F_1 plants were indistinguishable morphologically from *Phleum pratense*, as might be expected from the fact that this parent contributed 21 of the 28 chromosomes. (1) Prior to the initiation of flowering culms the hybrid plants had more basal leaves than the *P. pratense* parent (fig. 3), but since variability in this character occurs between pots of the same clone under greenhouse conditions, and since the comparison was between hybrid plants from seed and parental plants from clones, this difference may not be significant. (2) The hybrid plant maintained greater leaf growth during and following seed production than either parent. *P. pratense*, under both field and greenhouse culture, has sparse leaf growth at this stage, while *P. subulatum* is an annual and dies as soon as the seed is mature. (3) In the red coloring of the glumes and peduncles the hybrids were intermediate between *P. pratense*, which lacked such coloring, and *P. subulatum*, on which the glumes and peduncles were dark red, almost purple.

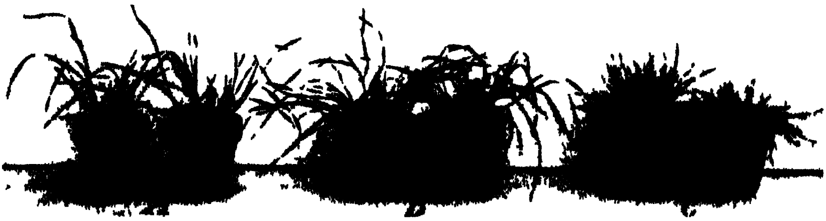

FIGURE 3 *A*, *Phleum pratense*, *B*, *P. pratense* × *P. subulatum*, *C*. *P subulatum*.

The hybrid plants were completely male sterile, as might be expected from their meiotic behavior. Young microspores rarely appeared normal, and the anthers were never observed to dehisce. Three panicles of the hybrid were enclosed under bags for selfing, but no seeds were set. Eight other panicles were pollinated with pollen from normal *P. pratense*, and 27 seeds, an average of 3.38 seeds per panicle, were obtained.

DISCUSSION

The bivalent frequency in the hybrid plants could be accounted for in 3 ways: (1) One genome of *Phleum pratense* is homologous with the genome of *P. subulatum*, and the other 2 genomes of *P. pratense* are partially homologous; (2) only partial homology exists between 1 genome of *P. pratense* and the genome of *P. subulatum*, while the other 2 genomes of *P. pratense* are completely homologous; (3) only partial homology exists between 1 *P. pratense* genome and the genome of *P. subulatum* as well as between the 2 remaining genomes of *P. pratense*. It is impossible from the data available to determine which of these explanations is the correct one. Some evidence for the first is that the maximum number of quadrivalents observed in *P. pratense* was 4, while the maximum number of bivalents in the hybrid was 12, or 5 more than would be accounted for by pairing between *P. pratense* and *P. subulatum* chromosomes.

On the basis of the results reported by Nordenskiöld (*18*) and Müntzing and Prakken (*13*), the second explanation seems more logical. However, if the hypothesis of complete homology between chromosomes of two genomes of *Phleum pratense* is accepted, it is necessary to account for the low frequency of quadrivalent formation in normal plants of this species. This question has been discussed thoroughly by Müntzing and Prakken (*13*). Little further discussion is warranted at present except to point out that in the plant of *P. pratense* used in this study the quadrivalent frequency was higher and the chiasma frequency lower than in the material used by Nordenskiöld (*18*) or by Müntzing and Prakken (*13*). Obviously, a more extensive study of plants of *P. pratense* is desirable.

Müntzing and Prakken (*13*) raised the question whether the chromosomes of the two homologous genomes of *Phleum pratense* normally associate as bivalents in particular pairs or whether bivalent formation was at random among each set of four homologues. Critical cytological evidence cannot be obtained on this question. On the other hand, the genetical consequences of the two types of pairing will be different. There is, however, a paucity of genetical data for *P. pratense*. Barker and Hayes (*1*) obtained 3:1 ratios for reaction to rust among inbred progenies, and Proytchoff (according to Horsfall (*6*)) obtained similar results. Likewise, Myers and Chilton (unpublished data), in studies of hybrid populations involving rust-resistant parents, obtained ratios of 3:1 in crosses of resistant × resistant, and ratios of 1:1 in crosses of resistant × susceptible plants. Unfortunately, this evidence is negative, and limited negative evidence from progenies of plants from open-pollinated populations is useless in solving this particular problem, since an allotetraploid of the constitution $A_1a_1a_2a_2$ will produce progenies in the same phenotypic ratios as will an autotetraploid of the constitution *Aaaa*.

The behavior of the univalents at metaphase I in the hybrid plants reported in this paper differs from the usual behavior of unpaired chromosomes. According to Darlington (*2*), Jenkin and Thomas (*7*), Levan (*8*), and others, univalents usually lie outside of the spindle at metaphase I and either do not congress or do so later than the bivalents. In the *Phleum pratense* × *P. subulatum* hybrid the univalents were lying in the equatorial plane along with the bivalents. In this respect the behavior resembles that reported by Levan (*8*) for the univalents in asynaptic *Allium amplectens*.

In his studies of a telocentric chromosome in maize, Rhoades (*20*) suggested that the univalents that congressed divided equationally at anaphase I. This conclusion is supported by the results obtained by the writer. In the hybrid reported in this paper, the univalents had congressed at metaphase I, and the average frequency of dividing univalents at anaphase I was almost equal to the average number of unpaired chromosomes at metaphase I. On the other hand, in *Lolium perenne*, Myers (*15*) found a lower frequency of dividing univalents at anaphase I than of unpaired chromosomes at metaphase I. In these plants the congression of univalents was not complete.

The occurrence of two dicentric bridges and acentric fragments at anaphase I in the hybrid plants indicates that at least two of the bivalents were heterozygous for an inversion. These inversions could have been present either in the bivalents resulting from pairing of *Phleum pratense* and *P. subulatum* chromosomes or in those resulting from autosyndesis of *P. pratense* chromosomes. In either case the presence of inversions does not necessarily represent a phase in the differentiation between the two species or between genomes of *P. pratense*. Müntzing (*12*), Myers and Hill (*16*, *17*), Myers (*15*), and Östergren (*19*) have reported the common occurrence of plants of normally cross-pollinated species that are heterozygous for inversions. It was not determined whether or not the *P. pratense* plant used in this investigation was heterozygous for one or more inversions.

SUMMARY

The 14 chromosomes of *Phleum subulatum* normally were associated as 7 bivalents at diakinesis and metaphase I. In 1.1 percent of the sporocytes 6 bivalents plus 2 univalents were present. The average chiasma frequency at metaphase I was 1.85 per bivalent. Anaphase I was regular, but at anaphase II some lagging univalents occurred, resulting in chromosomes being left in the cytoplasm in 2.47 percent of the quartets.

In *Phleum pratense*, at diakinesis, 33.3 percent of the sporocytes had 21 bivalents and 2.8 percent had 20 bivalents plus 2 univalents. The remaining 63.9 percent had 1 to 3 quadrivalents plus 19 to 15 bivalents. Four quadrivalents were observed rarely. The chiasma frequency was low, and about one-half of the bivalents had a chiasma in only 1 arm.

The hybrid plants (*Phleum pratense* × *P. subulatum*) had 28 chromosomes. At metaphase I the chromosomal association ranged from 7 bivalents plus 14 univalents to 12 bivalents plus 4 univalents, averaging 9.43 bivalents and 9.05 univalents per sporocyte. The univalents were oriented in a regular manner on the equatorial plane. Lagging univalents undergoing equational division were found in all anaphase I sporocytes, the average being 8.9. At metaphase II a majority of the daughter univalents congressed normally but did not divide at anaphase II. Laggards occurred at anaphase II in all sporocytes, resulting in irregular quartet formation.

Morphologically the hybrid closely resembled *Phleum pratense*. The hybrid was completely male sterile, since the anthers did not dehisce, but was partially female fertile with *P. pratense* pollen.

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GENETIC STUDIES OF VARIEGATION IN SNAP BEANS[1]

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INTRODUCTION

Since the introduction of Wisconsin Refugee and Idaho Refugee beans (*Phaseolus vulgaris L.*) in 1934 there have been many comments concerning variegation in these varieties (*4*)[2] [3] [4] and a partial genetic explanation of this or a related condition (*7*). The occurrence of variegations in certain breeding stocks at the United States Regional Vegetable Breeding Laboratory at Charleston, S. C., has made it necessary to give further attention to the problem of variegation.

MATERIAL AND METHODS

In the spring of 1936 a variegation occurred in a stock of U. S. No. 5 Refugee. This plant was undoubtedly of hybrid origin, because many of its characteristics were different from U. S. No. 5 Refugee, and its progeny segregated for seed color, pod color (wax and green), size of pods, and plant size. This material, which was of value for breeding purposes, was later used in crosses.

As variegation normally does not occur in U. S. No. 5 Refugee, it is possible that the variegated plant came from a mechanical mixture of some hybrids derived from crosses with strains of Corbett Refugee. Wisconsin Refugee and Idaho Refugee, which normally show variegation, were derived from crosses of Stringless Green Refugee with Corbett Refugee, and in many cases the hybrids carried by the United States Department of Agriculture at Greeley, Colo., showed variegation if Corbett Refugee was one of the parents.

The pure line of the variegated strain was crossed reciprocally with Black Valentine, and observations were made in the F_1, F_2, F_3, and F_4 generations. As previous experience with variegation in Corbett Refugee had shown that it was difficult to evaluate, the experiment was set up in such a way as to control variability as much as possible. The F_1 and F_2 plants were covered with cheesecloth at blooming time to shut out insects, and notes were taken at least three times during the life of a plant to be sure that the information obtained was accurate. F_1 plants were spaced 3 feet apart in 3-foot rows, F_2 seeds 1 foot apart, and F_3 and F_4 seeds 6 inches apart in 3-foot rows. The smallest F_3 family consisted of 39 plants and the average number per F_3 and F_4 family was over 60 plants.

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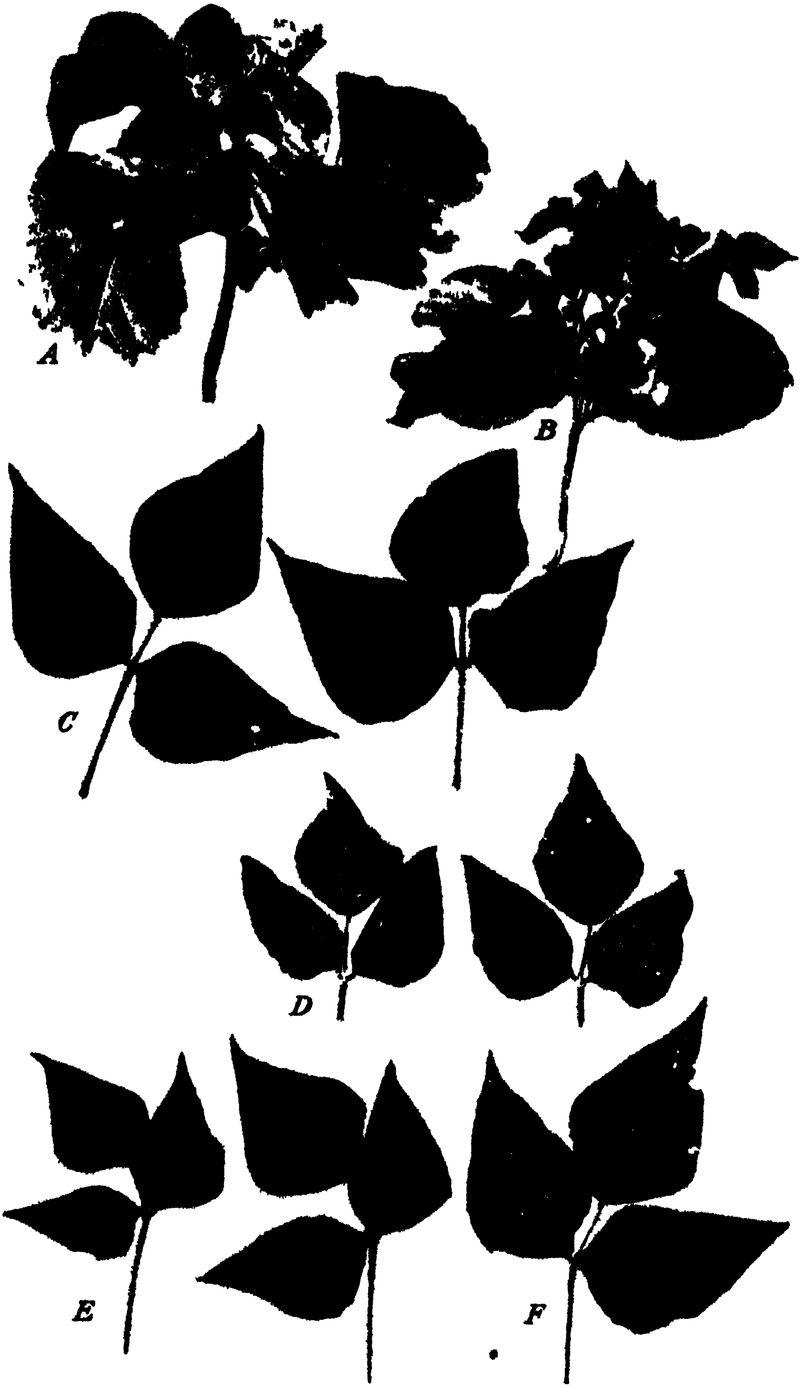

FIGURE 1 For explanatory legend see opposite page

All plants were field-grown except part of those grown to study the influence of environment on variegation (see table 3). The seeds for these tests were germinated on blotters and transferred to greenhouse soil as soon as roots had reached a length of about an inch. The plants were spaced 6 inches apart in rows 12 inches wide, and up to blooming time three sets of notes were taken on degree of variegation. In compiling the data the greatest degree of variegation noted for a plant was used.

The variegation discussed herein ranged from very severe (fig. 1, *A*) to very mild (fig. 1, *E*). Variegation rarely occurred in the primary leaves, but any trifoliate leaves produced up to blooming time might show it. After blooming the variegation tended to become less distinct, and only rarely did any leaves produced after blooming show it. The very severe type was not common, but the severe type was very common. The severe type differed from the very severe in that normal or nearly normal branches were put out by plants with the severe type and they produced normal crops, whereas the plants labeled very severe usually died before any pods were produced. In plants with the very severe and severe types the variegation ranged from a distinct chimera with deformity in some leaves to a mild flecking in other leaves (fig. 1, *A* and *B*). Plants of the medium, mild, and very mild types had one or more variegated leaflets, the variegation decreasing in intensity as shown in figure 1, *C* to *E*. Sometimes considerable deformity occurred, but usually only in the first two classes.

In all plantings the two parents were carried as checks. Because of the size of families it was not considered desirable to replicate, but the parents were planted at random and no effect of field location on expression of variegation was noticed.

All ratios were subjected to chi-square analysis according to the methods of Fisher (*1*), using Yates' correction for continuity, and of Mather (*3*), except that in testing for the significance of chi square beyond 30 degrees of freedom the approximation of Wilson and Hilferty (*6*) was used. Continuity corrections were applied to all ratios having 1 degree of freedom for chi-square computations, but for accumulated chi-square values (see tables 1 and 2) uncorrected values were added.

RESULTS

The F_1 plants from the cross of the variegated strain with Black Valentine and from the reciprocal showed no variegation. The F_1 plants were so prolific that only two plants were necessary to obtain sufficient F_2 seed.

From the F_2 plants 2 sets of results were recorded, 1 from the cross in which the variegated strain was used as the female parent (designated line A) and the other from the reciprocal (designated line B). Both lines segregated in a ratio of approximately 27 normal plants to

EXPLANATORY LEGEND FOR FIGURE 1.

FIGURE 1.—Types of variegation found under growing conditions favorable for the variegated parent: *A*, Very severe type; *B*, severe type; *C*, medium type, with very little deformity and most leaves with none; *D*, mild type; *E*, very mild type; *F*, normal (nonvariegated) leaf.

37 variegated. Table 1 shows the results from the two lines separately and in combination. The combined ratio is 293 normal to 369 variegated plants, with a nonsignificant chi-square value.

From the F_2 plants 220 of line A and 215 of line B were harvested for seed from the field as they occurred, without selection. From these were produced 124 and 122 variegated families of lines A and B, respectively. These 246 families were composed of all variegated plants except for 2 families from line A and 1 from line B, which had 1 or 2 plants each in which variegation could not be detected. These 5 plants were progeny-tested and found to be pure for the variegated condition.

From the 96 normal F_2 plants of line A were obtained 5 F_3 families in which all plants were normal, 25 with a ratio of 3 normal to 1 variegated, 40 with a ratio of 9 normal to 7 variegated, and 26 with a ratio of 27 normal to 37 variegated. From the 93 normal F_2 plants of line B were obtained 4 F_3 families in which all plants were normal, 22 with a 3:1 ratio, 38 with a 9:7 ratio, and 29 with a 27:37 ratio. Chi-square tests applied to these data for lines A and B, singly and combined, show no significant deviation from expectation for a 3-factor ratio of this kind (table 1).

Table 1.—*Results obtained with F_2 and F_3 progenies from the cross variegated × Black Valentine (line A) and the reciprocal (line B)*

[Degrees of freedom not indicated unless more than 1]

PARENTS

Line	Normal plants	Variegated plants
	Number	*Number*
Variegated	2	1,121
Black Valentine	1,063	[illegible]

F_2 GENERATION

Line	Normal plants	Variegated plants	χ^2 based on theoretical 27:37 ratio
	Number	*Number*	
A	159	180	2.900
B	134	189	.040
Total	293	369	1.082

F_3 FAMILIES [1]

Line	All normal	Segregating as indicated			All variegated	χ^2 based on theoretical 1:6:12:8:37 ratio	Degrees of freedom
		3:1	9:7	27:37			
	Number	*Number*	*Number*	*Number*	*Number*		*Number*
A	5	25	40	26	124	1.838	4
B	4	22	38	29	122	.634	4
Total	9	47	78	55	246	1.945	4

See footnotes at end of table.

Table 1.—*Results obtained with F_2 and F_3 progenies from the cross variegated × Black Valentine (line A) and the reciprocal (line B)*—Continued

F₃ SEGREGATING PROGENIES [4] [5]

Segregation ratio and line	Families	Accumulated χ^2	Degrees of freedom	Normal plants	Variegated plants	Population χ^2
3:1 ratio	*Number*		*Number*	*Number*	*Number*	
A	25	27 321	25	1,184	359	2 382
B	22	34 166*	22	960	260	8 657**
Total	47	61 487	47	2,144	619	9 609**
9:7 ratio						
A	40	38 850	40	1,510	1,053	6 815**
B	38	43 562	38	1,505	1,023	10 940**
Total	78	82 412	78	3,015	2 076	18 154**
27:37 ratio						
A	26	29 798	26	760	880	11 433**
B	29	33 608	29	837	1,016	6 636**
Total	55	63 406	55	1,597	1,896	17 727**

[1] Progeny test showed these to be variegated

[2] Progeny test showed this to be normal

[3] Separation into F_3 groups shown based on F_3 data, supplemented with F_4 data for all doubtful cases

[4] All F_3 progenies with chi square values in excess of 1 000 were studied in F_4

[5] * Significant at 5-percent level ** Significant at 1-percent level All heterogeneity chi squares (accumulated less population chi square) occur within the range of 5-percent to 95-percent probability. Continuity correction was applied to chi-square values with 1 degree of freedom, but added chi-square values of 1 degree of freedom each for accumulated chi square are uncorrected.

The F_3 segregating families when combined for chi-square tests for populations showed a deficiency of variegated plants. Chi square was highly significant in all cases except for the 3:1 families combined for line A (table 1).

The accumulated chi-square values for the various families indicate that families showing like segregations had been combined (table 1). Heterogeneity chi squares are not shown in the table, but in all cases when they are computed by subtracting chi square for the F_3 population from the corresponding accumulated chi square the values were within the range of 5-percent to 95-percent probability.

As ratios requiring large numbers for satisfactory tests are difficult to work with on account of the inability of many plants to set a large number of seeds, it was decided to give further attention to simpler tests requiring smaller numbers. Theoretically, in inheritance of the kind described in this paper there should be three types of variegated plants arising in connection with the 3:1 ratios. If two of the same type are crossed the F_1 plants should all be variegated and there should be no segregation in the F_2 generation, whereas if two of different types are crossed the F_1 plants should be normal and the F_2 segregation should be 9 normal to 7 variegated. Three types of variegated plants as postulated were recovered and tested in F_1 and F_2. All F_1 plants were normal. The F_2 results are given in table 2. All the ratios showed nonsignificant deficiency of variegated plants, but when the population chi square was computed it exceeded the 5-percent level of probability.

The three variegated types (*a*, *b*, and *c*) recovered from the 3:1 ratios were very difficult to distinguish from each other, but on the basis of the most fully variegated plants from each line they were tentatively described. Type *a* had a yellow tinge, type *b* was almost white in the variegated areas, and type *c* was intermediate or light yellow. Crosses between these types showed in the 9:7 F_2 ratios about the same range in amount of variegation as did the 3:1 ratios, but on an average the variegation covered a greater amount of leaf surface. The three 9:7 types of ratios obtained by crossing *a*, *b*, and *c* in all possible combinations were not distinguishable from each other. The 27:37 ratio gave variegated plants with somewhat greater variegation than did the 9:7 ratio. By taking advantage of this fact it was possible to distinguish in a preliminary way the 3:1, 9:7, and 27:37 families in F_3. By selecting for the most fully variegated plants in families segregating 27:37 it was possible to recover the triple recessive. This averaged heavily variegated with a slight yellowish tinge, and in crosses with types *a*, *b*, and *c* it always produced a variegated F_1 and no segregation in F_2. On the basis of variegation it could not be distinguished from the original variegated parent.

TABLE 2.—*Variegated and normal F_2 plants from crossing three types (a, b, and c) of variegated F_3 segregates from a cross of Black Valentine × variegated*

Cross number and variegation types crossed	Normal plants	Variegated plants	Chi square based on theoretical 9:7 ratio
	Number	*Number*	
1 (*a*×*b*)	104	76	0.171
2 (*b*×*a*)	94	62	1.018
3 (*a*×*c*)	106	65	2.288
4 (*c*×*a*)	84	60	.254
5 (*b*×*c*)	84	54	1.197
6 (*c*×*b*)	102	71	.516
Total accumulated χ^2			5.444
Total population χ^2	574	388	[1] 4.427*
Heterogeneity χ^2 for 5 degrees of freedom			[2] 1.017

[1] * = Significant at 5 percent level of probability.
[2] 95-percent level of probability = 1.145.

As the expression of variegation fluctuated so much, it was decided to study this under various conditions with selected strains. Sufficient seed of each strain was planted to insure at least 50 variegated plants in plantings made in the field in both early and late spring and in the greenhouse. Deformity of leaves was disregarded, and a scale of values from 1, with only a trace of variegation, up to 20, with about 25 percent of the total leaf surface variegated, was used. The parents, the recovered triple recessive, Corbett Refugee, and variegated plants from 3 lines of 3:1 and from 3 lines of 9:7 were observed. The best expression of variegation occurred under greenhouse conditions with temperatures between 70° and 80° F.; the next best occurred in the field under early-spring conditions (table 3). Late-spring planting in the field resulted in a reduction of the range as well as of the mean. Corbett Refugee was observed to be the most variable in expression of variegation. In field plantings of Corbett Refugee only about 25 percent showed variegation, and this was relatively slight. Under greenhouse conditions, however, all Corbett Refugee plants were variegated, and the mean was very close to the mean of the 3 lines carrying double recessives.

TABLE 3.—*Variegation in various types of variegated plants grown in three different environments*

[Variegation scale of 1 for trace to 20 for variegation on about 25 percent of leaf surface, 50 variegated plants of each strain]

Strain	Degree of variegation in—					
	Field planting in—				Greenhouse planting	
	Early spring		Late spring			
	Range	Mean	Range	Mean	Range	Mean
Black Valentine	0	0	0	0	0	0
Variegated (original parent)	1-19	12.0	1-12	7.0	2-20	14.3
Line of type *a* from 3:1 ratio	1-18	7.1	1-11	5.0	2-20	10.2
Line of type *b* from 3:1 ratio	2-18	7.4	1-11	5.3	2-20	10.1
Line of type *c* from 3:1 ratio	1-19	7.0	1-10	5.1	2-20	10.0
Triple recessive	1-19	12.4	1-12	7.5	2-20	14.0
Variegated from 9:7 ratio (*a*×*b*)	1-18	9.5	1-10	6.4	2-20	11.8
Variegated from 9:7 ratio (*a*×*c*)	1-18	9.0	1-11	6.0	2-20	11.6
Variegated from 9:7 ratio (*b*×*c*)	2-19	9.3	1-11	6.2	2-20	11.2
Corbett Refugee [1]	1-7	3.0	1-5	2.3	2-15	11.3
Significant difference		2.0		2.3		1.1

[1] Data for Corbett Refugee are based only on plants showing variegation (about 25 percent of plants in field, 100 percent of plants in greenhouse)

As over 26,000 plants were observed in the F_3 generation alone, it is reasonable to inquire to what extent misclassifications may have occurred. Table 1 shows that of 1,123 plants of the variegated parent line, 2 failed to show variegation, but all the progeny produced from these 2 plants were variegated. Of a total of 1,064 Black Valentine plants, 1 plant showed a slight loss of chlorophyll suspiciously like a mild variegation, but all the offspring were normal. If these errors tend to balance each other, then there is an error of classification of about 0.1 percent, or about 26 plants out of the 26,000 observed. Three F_3 of 246 variegated families gave 5 nonvariegated plants, all of which produced variegated progeny. Since the 246 families involved had approximately 15,000 plants, 15 plants would have been expected to be misclassified on the basis of 0.1 percent.

No natural crosses were observed except in the F_4 group of material, indicating that covering F_1 and F_2 plants with cheesecloth at blooming time had been effective in shutting out pollen-carrying insects.

DISCUSSION

The variegation reported here fluctuates a great deal in its expression, and no reason for this has been found. Despite this fluctuation, it has been possible to arrive at a factorial explanation. The normal condition is due to the complementary action of three dominant genes, and the variegation is due to the action of any of three recessive genes.

No evidence has been presented to show the relationship of the Corbett Refugee type of variegation to that reported, and attempts to make crosses of Corbett Refugee with the variegated types have failed in both the greenhouse and the field at Charleston, S. C. However, there is the probability that the variegation found in Corbett Refugee and in U. S. No. 5 Refugee came originally from the same source, since over a period of years U. S. No. 5 Refugee had not previously yielded any variegation, and many lines of beans involving variegations derived from Corbett Refugee crosses were being carried at Greeley, Colo., at the time when the U. S. No. 5 Refugee, which later produced the variegation, was grown there. On many occasions it has been observed that crosses between Corbett Refugee and other varieties (notably Stringless Green Refugee and Brittle Wax) have given rise to progenies with very distinct variegation.

Variegations and chimeras have been found to behave variously in genetic studies, many of them being due more or less to plasmatic inheritance. These conditions have recently been reviewed by Sirks (*5*) and Jones (*2*).

Various seedsmen and breeders have reported that variegations in lines derived from Corbett Refugee behave peculiarly from a breeding standpoint and that from lines carrying chimeras they have been unable to isolate lines breeding true for the normal nonvariegated type. If the condition they reported is essentially the same as the one described in this paper and if they were selecting from an F_2 segregating population they could have expected only 1 plant out of 64 to be a pure normal; many of the variegations would have been obscured, since seedsmen normally make their selections 2 or 3 weeks after blooming time, when variegation is very difficult to detect.

It is suggested that seedsmen working with crosses involving such a variegation should make their observations several times during the growth of the plants so as to make sure that the plants selected do not show any variegation at any time. It is also suggested that many selections be made, probably a few hundred rather than a few dozen, in order to make sure that the desired normal type is selected.

CONCLUSIONS AND SUMMARY

The variegation treated in this paper is due to any one of three recessive factors, whereas the normal condition is due to the complementary action of three dominant genes giving in F_2 a ratio of 27 normal to 37 variegated for the cross of Black Valentine with the original variegated strain and the reciprocal cross. This was verified from F_1, F_2, and F_3 data as well as from some F_4 data for certain variable F_3 families. Intercrosses of the three types of variegated obtained from F_3 families segregating in 3:1 ratios gave nonvariegated F_1 plants and ratios of 9 normal to 7 variegated in F_2. These same three monofactorial variegated types crossed with the triple recessive gave all variegated in F_1 and F_2 generations.

The variegation is expressed over a wide range, from only a trace up to a very severe, semilethal condition. The range is approximately the same for variegated lines due to one, two, or three factors, but the average amount of variegation for three factors is more than for one or two, and the average for two more than for one. The relative order remained the same under favorable growing conditions in the field, under unfavorable conditions in the field, and under favorable growing conditions in the greenhouse. The best expression of variegation was obtained under greenhouse conditions, and the poorest under unfavorable growing conditions in the field.

Altogether 662 F_2 plants and 435 F_3 families were studied; the 180 F_3 segregating families consisted of 11,347 plants. In addition, some test crosses of various segregation types and some F_3 families were carried to F_4. The plants and families were about equally divided between the cross and its reciprocal. F_3 population chi squares indicated a significant deficiency of recessives in nearly all cases. This is to be expected, since in the very severe form the variegated condition does not have as great survival value as do the normal plants. Heterogeneity chi-square values indicated that in all cases homogeneous populations were combined for the F_3 chi-square tests.

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A MOSAIC DISEASE OF PRIMULA OBCONICA AND ITS CONTROL [1]

By C. M. Tompkins, *assistant plant pathologist*, and John T. Middleton, *junior plant pathologist, California Agricultural Experiment Station* [2]

INTRODUCTION

A mosaic disease of *Primula obconica* Hance, grown extensively as a potted ornamental plant in commercial greenhouses in San Francisco, was first observed in August 1937. The incidence of the disease on very young to older seedling plants ranged from 5 to 25 percent and, because infected plants could not be marketed, serious financial losses were incurred.

The results of studies on transmission, experimental host range, and properties of the virus, as well as control of the disease, are presented in this paper.

REVIEW OF LITERATURE

That many cultivated species of the genus *Primula* are generally susceptible to virus infection is indicated in the literature. In addition to the mosaic diseases, the aster yellows, curly top, spotted wilt, and certain tobacco viruses may cause infection. Since this paper deals with a mosaic disease, only pertinent references are listed.

In Japan, Fukushi (*4*)[3] in 1932 and Hino (*6*) in 1933 recorded the occurrence of a mosaic disease on *Primula obconica*. The latter also observed a similar disease on *P. denticulata* Sm.

Smith (*13*) in England, in 1935, described a virus disease of the mosaic type on *Primula obconica* caused by cucumber virus 1. Later, Smith (*14*) stated that *Cucumis* virus 1 sometimes caused color breaking of the flowers and that other species of *Primula* were susceptible. *Cucumis* virus 1B, a strain, induced "a pronounced yellow and green mottling."

In Germany, Ludwigs (*7*) and Pape (*8*) described very briefly a virus disease of *Primula obconica* without apparently establishing the identity of the virus.

There are numerous brief references dealing with mosaic diseases of *Primula* spp. grown in greenhouses and out of doors, but the viruses were not identified. In 1926, Anderson et al. (*2*) mentioned the occurrence of a mosaic disease of *Primula* sp. in a Pennsylvania greenhouse, while Martin[4] recorded a similar disease affecting several hundred plants in a Michigan greenhouse which resulted in a total loss to the grower. Gram (*5*) and Yu (*16*) found mosaic-infected primroses grown out of doors. Flachs (*3*), White (*15*), and Pirone (*9*) also listed primroses as susceptible to mosaic-virus infection, but it is not clear whether they referred to species grown in greenhouses or in the field.

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[1] Received for publication February 4, 1941

[2] The assistance of nontechnical employees of the Federal Work Projects Administration Project No. 65 1-08-91 is acknowledged

[3] Italic numbers in parentheses refer to Literature Cited, p 679

[4] MARTIN, G HAMILTON DISEASES OF FOREST AND SHADE TREES, ORNAMENTAL AND MISCELLANEOUS PLANTS IN THE UNITED STATES IN 1925 U S Bur Plant Indus, [Mimeographed] Plant Dis Rptr Sup 50 413 478, illus 1926

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Under experimental conditions, Price (*10*) succeeded recently in transferring the alfalfa mosaic virus to *Primula obconica*. A description of the symptoms was not given.

SYMPTOMS OF THE DISEASE

Characteristic symptoms of the mosaic disease of *Primula obconica* plants are best expressed in greenhouses having a humid atmosphere

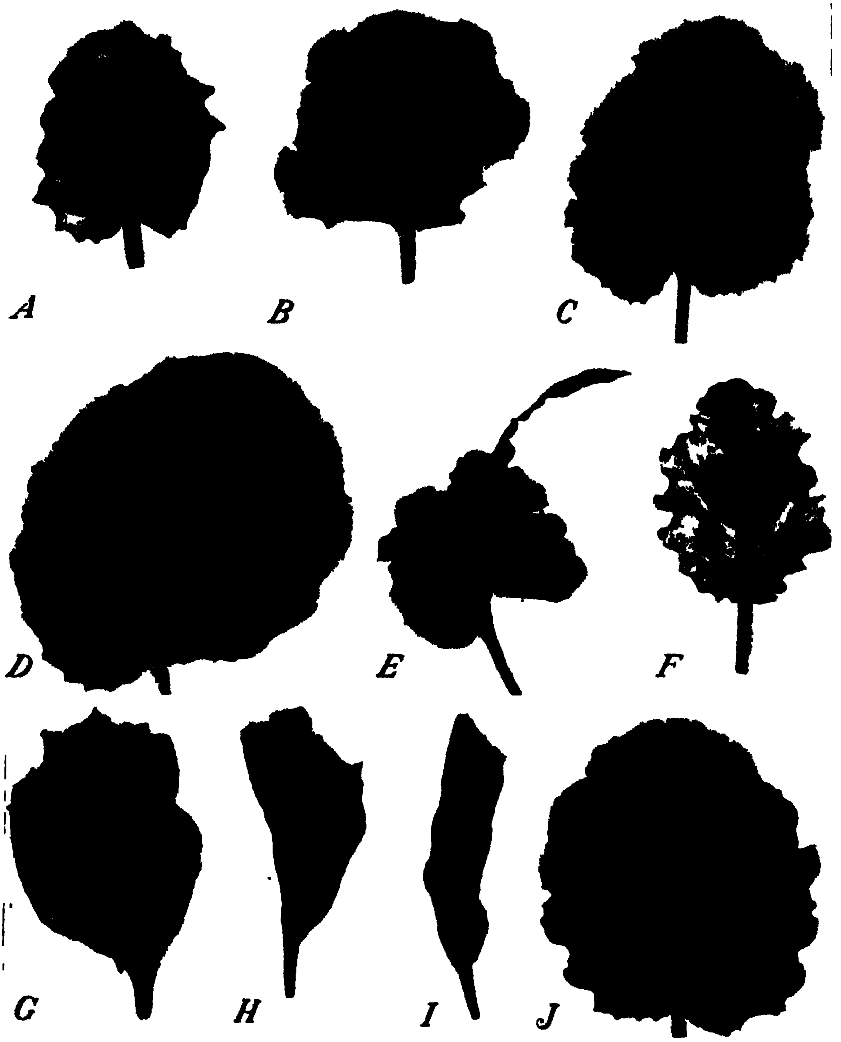

FIGURE 1.—Symptoms produced by a mosaic virus of *Primula obconica* on leaves of healthy *P. obconica* plants after mechanical inoculation in the greenhouse at 65° to 70° F.: *A*, *B*, Early symptoms, consisting of a systemic, yellow-green mottle; *C*, numerous, small, circular to irregular, raised, dark-green islands frequently occur between and adjacent to the veins on a mottled background; *D*, coarse, yellow-green mottle on leaf with flat, dark-green islands; *E*, a shoestring effect is sometimes produced, in addition to mottling and dark-green islands; late symptoms, consisting of *F*, rugosity of the leaves; *G*, *H*, *I* cupping or upward rolling of the leaves; *J*, noninoculated control (healthy).

and air temperatures ranging from 65° to 70° F. Although the disease may affect young to middle-aged plants, it appears most commonly in young potted plants (three to five leaves). The first symptom consists of a systemic, coarse, yellow-green mottle of the leaves (fig. 1, *A*, *B*). With enlargement of the leaf area, the mottle becomes more conspicuous, consisting of numerous, dark-green, circular to irregular, raised islands (fig. 1, *C*), ranging in size from a mere fleck to 3 to 5 mm. in diameter, which are distributed between and adjacent to the veins and surrounded by nonraised or flat, light-green to yellow mottled areas (fig. 1, *A*, *B*). Infrequently, leaves of some infected plants show a coarse mottle with flat islands (fig. 1, *D*). Occasionally, also, the virus induces a shoestring effect at or near the tip of the leaf (fig. 1, *E*). These effects are in marked contrast to the appearance of a healthy leaf (fig. 1, *J*).

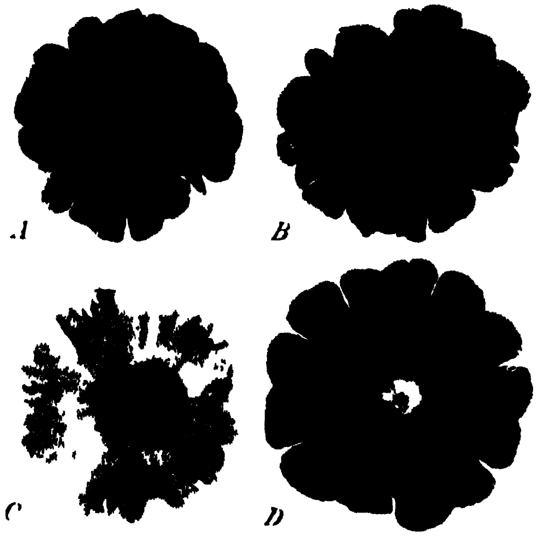

FIGURE 2 Symptoms produced by a mosaic virus of *Primula obconica* on flowers of healthy *P. obconica* plants after mechanical inoculation in the greenhouse at 65° to 70° F. *A*, *B*, *C*, Color breaking of flowers as indicated by irregular-shaped, white streaks interspersed among areas of normal pigmentation; *D*, noninoculated control (healthy)

With the onset of the disease, infected plants show a general chlorosis, marked stunting, and generally rugosity (fig. 1, *F*), with an upward curling or cupping of the leaves (fig. 1, *G*, *H*, *I*). Sometimes the cupping is so extreme that the edges of the leaf touch each other over the midrib. Occasionally the reverse condition holds, in that the center of the leaves becomes arched and the edges curl downward. The petioles and peduncles of infected plants are greatly shortened, while the flowers are usually much reduced in size. The petals show a conspicuous color breaking or variegation as indicated by irregular-shaped, white streaks interspersed among areas of normal pigmentation, which are more distinct near the base of the petals than at the edge (fig. 2, *A*, *B*, *C*), in contrast to the normal, healthy condition (fig. 2, *D*). The most noticeable color breaking occurs on deep pink or lilac flowers, and frequently the pigments are almost totally destroyed. On lighter shades, it is difficult to detect any color change. Another symptom is a distinct, coarse mottle of the calyx. When infected plants reach maturity, they have usually only attained about one-fourth the normal size and cannot be marketed (fig. 3, *A*).

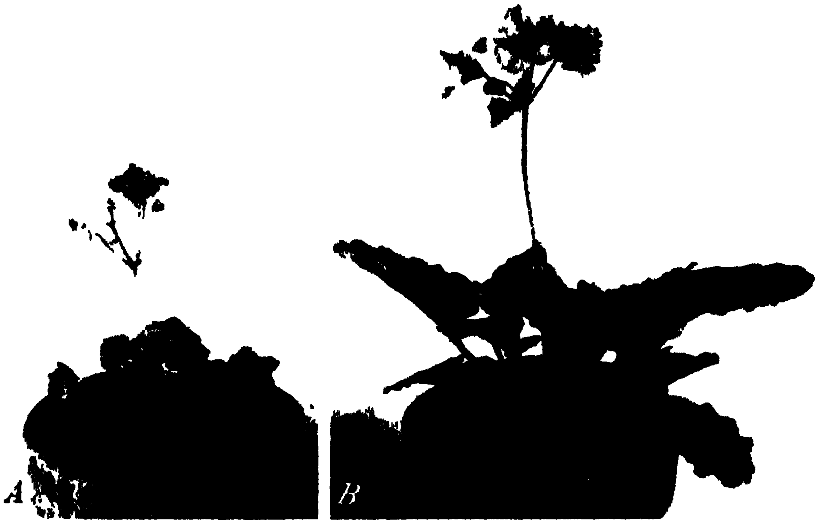

FIGURE 3.—Symptoms produced by a mosaic virus of *Primula obconica* on leaves of a healthy *P. obconica* plant after mechanical inoculation in the greenhouse at 65° to 70° F.: *A*, Diseased plant, showing dwarfing, mottling, and upward rolling of leaves and dwarfing of flowers; *B*, noninoculated control (healthy)

MATERIALS AND METHODS

Naturally infected *Primula obconica* seedlings were collected in a greenhouse in San Francisco, Calif., from which the mosaic virus used in these studies was obtained. The virus was transferred to healthy *P. obconica* plants, and transfers were made at monthly intervals. This plant species served also as the standard test plant for virus-recovery, virus-property, and insect-transmission studies.

All inoculations were made in a greenhouse where temperatures ranged from 65° to 70° F. Mechanical or juice inoculations were made by dusting the leaves with 600-mesh, powdered carborundum (*11*) and lightly rubbing with absorbent cotton dipped in juice from a diseased plant.

TRANSMISSION

The incubation period, as determined by numerous serial transfers of the virus by mechanical inoculation to healthy *Primula obconica* plants, ranged from 16 to 21 days.

Studies on insect transmission of this disease involved the testing of several species of aphids which are known to occur in local greenhouses, including the green peach aphid (*Myzus persicae* Sulzer) and the lily aphid (*Myzus circumflexus* Buckton). Noninfective aphids were permitted to feed for 24 to 48 hours on infected plants and were then transferred to healthy plants. All attempts to transmit the virus by means of aphids were unsuccessful.

In excess of 20,000 *Primula obconica* seedlings (one to two leaves) in flats have been examined over a period of 3 years for evidence of virus infection, but with negative results. It must be assumed, therefore, that the virus is probably not seed-borne.

EXPERIMENTAL HOST RANGE

By means of mechanical inoculations, the *Primula obconica* mosaic virus was transmitted to two species within the same genus, namely, fairy primrose (*P. malacoides* Franch.) and Chinese primrose (*P. sinensis* Lindl.) and was later recovered from all infected plants. Infection was not obtained on any of the numerous species of plants belonging to different families as indicated by lack of visible symptoms on inoculated plants and by failure to recover the virus from them on healthy *P. obconica* plants. Numerous series of inoculations were made to Turkish tobacco (*Nicotiana tabacum* L.) and *N. glutinosa* L. with negative results.

This virus is apparently limited to the genus from which it was originally isolated. Somewhat analogous instances reported within recent years may be cited. According to Ainsworth (*1*), the green-mottle mosaic of cucumber (cucumber virus 3) and the yellow mosaic of cucumber (cucumber virus 4) occur in nature only on cucumber and are not transmissible to solanaceous plants. Severin and Freitag (*12*) succeeded in transferring the western celery mosaic virus only to species within the family Umbelliferae.

No infection was obtained by mechanical inoculation with the *Primula obconica* mosaic virus in 46 species of plants representing 42 genera in 23 families, as follows:

- Begoniaceae:
  - Fibrous-rooted begonia (*Begonia semperflorens* Link and Otto) var. Fire Sea
- Boraginaceae:
  - Forget-me-not (*Myosotis alpestris* Schmidt)
- Campanulaceae:
  - Canterbury bells (*Campanula medium* L.)
- Caryophyllaceae:
  - Sweet-william (*Dianthus barbatus* L.)
- Chenopodiaceae:
  - Spinach (*Spinacia oleracea* L.) var. Bloomsdale
- Compositae:
  - Romaine lettuce (*Lactuca sativa* L. var. *longifolia* Lam.)
  - English daisy (*Bellis perennis* L.)
  - China-aster (*Callistephus chinensis* Nees) var. Giant Branching White, wilt resistant
  - French marigold (*Tagetes patula* L.)
  - Transvaal daisy (*Gerbera jamesonii* Hook.)
  - Hybrid cineraria (*Senecio cruentus* DC.)
- Cruciferae:
  - Chinese cabbage (*Brassica pe-tsai* Bailey)
  - Turnip (*B. rapa* L.) var. Purple Top White Globe
  - Cabbage (*B. oleracea* L. var. *capitata* L.) var. Winter Colma
  - Cauliflower (*B. oleracea* var. *botrytis* L.) var. February
  - Radish (*Raphanus sativus* L.) var. White Icicle
  - Annual stock (*Matthiola incana* R. Br. var. *annua* Voss) var. Fiery Blood Red

- Cucurbitaceae:
  - Cucumber (*Cucumis sativus* L.) var. Arlington White Spine
  - Pumpkin (*Cucurbita pepo* L. var. *condensa* Bailey) var. Zucchini
- Euphorbiaceae:
  - Castor-bean (*Ricinus communis* L.)
- Gramineae:
  - Corn (*Zea mays* L.) var. Golden Bantam
- Leguminosae:
  - Broadbean (*Vicia faba* L.)
  - Cowpea (*Vigna sinensis* (Torner) Savi)
  - Garden pea (*Pisum sativum* L.) var. Alderman
- Lobeliaceae:
  - Lobelia (*Lobelia hybrida* Hort.)
- Papaveraceae:
  - Oriental poppy (*Papave orientale* L.)
- Primulaceae:
  - *Primula auricula* L.
  - Cowslip (*P. veris* L.)
  - Florists' cyclamen (*Cyclamen indicum* L.)
  - Poor-man's-weatherglass (*Anagallis arvensis* L.)
- Ranunculaceae:
  - Candle larkspur (*Delphinum cultorum* Voss)
  - Persian buttercup (*Ranunculus asiaticus* L.)
  - Poppy anemone (*Anemone coronaria* L.)
- Resedaceae:
  - Mignonette (*Reseda odorata* L.)
- Rosaceae:
  - *Geum chiloense* Balb.
- Scrophulariaceae:
  - Snapdragon (*Antirrhinum majus* L.)
  - Pentstemon or beardtongue (*Pentstemon barbatus* Nutt.)
- Solanaceae:
  - Potato (*Solanum tuberosum* L.)
  - Tomato (*Lycopersicum esculentum* Mill. var. *vulgare* Bailey) var. Early Santa Clara Canner
  - Jimsonweed (*Datura stramonium* L.)
  - Bell pepper (*Capsicum frutescens* L. var. *grossum* Bailey) var. California Wonder
  - *Nicotiana glutinosa* L.
  - Tobacco (*N. tobacum* L.) var. Turkish and White Burley
- Tropaeolaceae:
  - Garden nasturtium (*Tropaeolum majus* L.)
- Umbelliferae.
  - Celery (*Apium graveolens* L.) var. Golden Self Blanching
- Verbenaceae:
  - Garden verbena (*Verbena hybrida* Voss)
- Violaceae:
  - Pansy (*Viola tricolor* L.)

PROPERTIES OF THE VIRUS

The results of property studies of the *Primula obconica* mosaic virus are shown in table 1. Virus samples, consisting of 2 cc. of undiluted juice in small, thin-walled test tubes, were used in determining resistance to aging in vitro and inactivation temperature. In determining the tolerance to dilution, the virus was diluted with the required amount of distilled water. Young *P. obconica* plants were then inoculated mechanically with the different virus samples.

Samples of the *Primula obconica* mosaic virus were infectious after 24 hours aging in vitro at 22° C., but were inactivated after 48 hours. The virus produced infection after heating for 10 minutes at 48°, but was inactivated at 50°. A dilution tolerance of 1 to 10 was established. In each of the four trials, 16 noninoculated *P. obconica* plants served as controls and they remained healthy.

TABLE 1. *Longevity in vitro, inactivation temperature, and tolerance to dilution of the Primula obconica mosaic virus*

[4 trials made with 16 plants each in all instances]

LONGEVITY IN VITRO, 22° C

Aged (hours)	Plants infected	Aged (hours)	Plants infected	Aged (hours)	Plants infected	Aged (hours)	Plants infected
0	16	6	1	24	2	72	0
3	10	8	3	48	0	96	0

INACTIVATION TEMPERATURE (10 MINUTES)

Temperature (°C)	Plants infected	Temperature (°C)	Plants infected	Temperature (°C)	Plants infected	Temperature (°C)	Plants infected
40	15	48	14	55	0	65	0
45	12	50	0	60	0	[1]	16

TOLERANCE TO DILUTION

Dilution	Plants infected	Dilution	Plants infected	Dilution	Plants infected	Dilution	Plants infected
0	14	1:100	0	1:1,000	0	1:5,000	0
1:10	8	1:500	0	1:2,000	0	1:1,000	0

[1] Untreated

DESCRIPTION OF THE PRIMULA OBCONICA MOSAIC VIRUS

Transmissible by mechanical inoculation with carborundum. Incubation period 16 to 21 days. Resistance to aging in vitro between 24 and 48 hours. Inactivation temperature between 48° and 50° C for a 10-minute exposure. Tolerance to dilution approximately 1 to 10. Two additional species of *Primula* susceptible: *P. malacoides* and *P. sinensis*. Experimental host range apparently confined to this genus.

COMPARISON OF THE PRIMULA OBCONICA MOSAIC VIRUS WITH CERTAIN OTHER VIRUSES

As previously mentioned, Smith (*13*) has described a virus disease of *Primula obconica* in England caused by cucumber virus 1. During the course of the present studies, parallel series of inoculations were made simultaneously to healthy *P. obconica* plants with ordinary cucumber mosaic virus supplied by Dr. James Johnson of the Wisconsin Agricultural Experiment Station; an undescribed cucumber mosaic virus which occurs naturally in California; celery calico virus (*12*); and the mosaic virus of *P. obconica*[5] in order to determine whether the latter virus was similar to or identical with any of the aforementioned.

Ordinary cucumber mosaic virus and the undescribed California cucumber mosaic virus readily infected young *Primula obconica* plants, both inducing systemic infection. Symptoms caused by cucumber virus 1 consisted of a coarse mottle, with slight, upward cupping of the leaves, and a mild type of flower breaking. With the western cucumber mosaic virus, a coarse mottle, accompanied by large, irregular-shaped, raised, dark-green islands, and severe flower breaking, occurred. In neither case were infected plants visibly retarded in growth. No infection resulted from inoculations with the celery calico virus. Considered as to symptoms, there can be little or no difficulty in differentiating between the mosaic virus of *P. obconica* and the other two viruses which infected this host.

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[5] The cucumber and celery viruses were kindly supplied by Drs. H. H. P. Severin and J. H. Freitag, Division of Entomology and Parasitology, University of California.

Other plants which were tested with the four viruses include celery, cucumber, spinach, tomato, Turkish tobacco, and *Nicotiana glutinosa*. Again the *Primula obconica* mosaic virus failed to infect these plants, whereas infection was readily obtained with the other viruses.

As a result of these tests, it is concluded that the virus described in this paper is different from the three viruses with which it has been compared and the virus of *Primula obconica* described by Smith (*13*).

CONTROL OF THE DISEASE

Primula obconica plants showing mosaic infection were found on benches in different parts of the San Francisco greenhouses under observation, indicating a spread of the disease by some unknown insect vector. Attempts to locate other susceptible greenhouse plants which might serve as virus reservoirs and aphids which might conceivably act as vectors were unsuccessful.

In order to reduce, if not eliminate, further loss to growers as soon as possible, careful roguing of disease plants was practiced, and the greenhouses were fumigated weekly with nicotine dust. The incidence of the disease was quickly reduced, until at present it is believed that the disease has been entirely eradicated in these houses.

SUMMARY

A mosaic disease of *Primula obconica*, prevalent in greenhouses in San Francisco, is described.

The symptoms of the disease include a prominent leaf mottle, consisting of irregular-shaped, dark-green, raised or nonraised islands on a light-green to yellow background, with upward curling or cupping of the leaves. Occasionally a shoestring effect is induced at or near the tip of the leaf. The leaves, flowers, petioles, and peduncles are reduced in size. The petals of infected flowers show a conspicuous color breaking or variegation, while the calyx is mottled. Usually infected plants are severely stunted.

The *Primula obconica* mosaic virus is readily transmissible by juice inoculation, with carborundum. The incubation period ranges from 16 to 21 days. The green peach and lily aphids failed to transmit the virus under greenhouse conditions.

The virus retained its infectivity after aging for only 24 hours at 22° C. It is inactivated by heating for 10 minutes at 50° and has a dilution tolerance of 1 to 10.

The host range of the *Primula obconica* mosaic virus is limited to two additional species within the same genus, namely, *P. malacoides* and *P. sinensis*.

The disease has been eradicated by careful roguing of diseased plants and by frequent fumigation of the greenhouses.

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RELATION BETWEEN WETTING POWER OF A SPRAY AND ITS INITIAL RETENTION BY A FRUIT SURFACE[1]

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INTRODUCTION

The wetting power of a spray solution is a very important factor in its efficiency as a contact poison or as a stomach poison. In the field of contact sprays this has been clearly recognized by a number of workers (*1, 8, 9, 11*)[2], who have presented logical explanations of their results and of the physicochemical phenomena involved. In earlier papers (*2, 3, 4, 5*) the author has summarized some of this work and has developed the use of the spreading coefficient on mineral oil as a convenient quantitative measure of wetting power. The spreading coefficient, S, of an aqueous solution with respect to a mineral oil is defined by the equation $S = T_B - T_A - T_{AB}$, where T_B is the surface tension of the oil, T_A is the surface tension of the aqueous solution, and T_{AB} is the interfacial tension.

In the field of stomach poisons and protective fungicides, which function by means of the deposit that remains after evaporation of the liquid medium, the factors involved have been less clearly recognized. Working with oil-emulsion sprays, Smith (*10*) has found that increased amounts of emulsifier produce sprays which wet the surfaces better but which deposit less oil, and he has advanced a working hypothesis to explain this phenomenon. However, his explanation does not clearly point out, or take into account, the fact that when the spray mixture wets the surface poorly a much greater volume may be retained on the sprayed surface. This factor alone may account for the observed increase in the deposition of insecticide when little or no emulsifier is used. The author (*5*) has briefly commented on the factors involved in the application of spray mixtures of this type and has pointed out that maximum efficiency may be associated with relatively high surface and interfacial tensions, i. e., with a low spreading coefficient. Fajans and Martin (*6, 7*) have recently reported work in this field, in which they have determined solid-liquid contact angles as a measure of wetting power.

Statements are occasionally made that an excess of wetter causes excessive run off and a reduced deposit of insecticidal material on the fruit and foliage, or, conversely, that the addition of a wetter produces better wetting and adherence and an increased deposit of insecticidal material. Obviously, in any given case many factors are involved besides the specific nature of the surface to which the spray is being applied. Such factors include the wetting agent, the influence of other constituents of the spray mixture, and the water supply. The object of the present work has been to determine whether there is a definite relation between the wetting power of

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[2] Italic numbers in parentheses refer to Literature Cited, p. 685.

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the spray mixture, as measured by its spreading coefficient on mineral oil, and the volume of spray mixture retained by fruit and foliage. If so, the problem can possibly be simplified to the consideration of but two factors, (1) the surface being sprayed and (2) the spreading coefficient of the spray mixture.

EXPERIMENTAL METHOD

The general plan of the experiments was to rotate an apple, while spraying it with a spray solution of known spreading coefficient, until run-off commenced, and then to determine the volume of the spray solution retained on the apple. Variation in wetting power was produced by the addition of different amounts of a wetting agent, and the spreading coefficients were determined as previously described (*5*). Two series of tests were made in which two distinct methods for determining the volume of retained spray solution were used. An interval of several months separated the two series. As the spreading coefficient associated with a definite retention would be expected to vary with the type of fruit, its maturity, and possibly other factors, these experiments are regarded as illustrative only.

For rotating the apple during the spraying the stem was tied to a string dropped from the vertical shaft of a variable-speed laboratory stirrer, which was operated at about 30 revolutions per minute. The spray solution was applied with a small lacquer-spraying nozzle attached to the laboratory air supply, which was kept at a constant distance from the apple and was manipulated in as nearly uniform a manner as possible. The spray thus produced was a rather fine mist, and it is possible that a coarse, driving spray would give somewhat different results.

TESTS WITH SPRAYS CONTAINING SODIUM BICARBONATE AND WETTING AGENT

In the first series of tests the spray liquid was a solution of sodium bicarbonate, approximately N/2, containing varying amounts of a wetting agent (No. 1). Measurements were made of the surface tension and the spreading coefficient on mineral oil of most of the spray solutions. With the apple rotating, spraying was begun and continued until a distinct run-off occurred. Spraying was then discontinued, rotation of the apple stopped, and the spray solution retained by the apple was quickly washed into a beaker with distilled water. The sodium bicarbonate thus recovered from the apple was titrated with standard hydrochloric acid, methyl orange being used as indicator.

The titer of the spray mixture being known, a simple calculation gave the volume of spray solution retained by the apple, upon the assumption that there was no concentration of the solution by evaporation. Each of the uncorrected values thus obtained was multiplied by the constant factor 0.55, to make an approximate correction for the increase in concentration of the solution due to evaporation during the spraying process. This factor was determined by weighing the apple before and after spraying with a mixture containing no wetting agent and comparing the volume of retained spray thus found with the volume found by titration.

Table 1 and figure 1 present the results of these experiments, together with the calculated values of the relative retention, which is here defined as the ratio of the volume of spray retained to the volume retained when the spray liquid is distilled water.

TABLE 1 *Variation of wetting properties and initial retention with the concentration of wetter No 1 in an approximately N/2 solution of sodium bicarbonate*

Concentration of wetter		Spray retained		Relative retention	Surface tension	Interfacial tension	Spreading coefficient
		Titration	Corrected				
Percent by weight	*Log of grams per cubic meter*	*Cubic centimeters*	*Cubic centimeters*		*Dynes per centimeter*	*Dynes per centimeter*	*Dynes per centimeter*
1 000	4 000	0 51	0 28	0 14	29 2	1 1	+0 2
500	3 699	57	31	16			
250	3 398	50	26	14			
125	3 097	58	32	16	29 2	7	+ 6
0625	2 796	58	32	16	28 8	8	+ 9
0313	2 496	61	35	17			
0156	2 193	83	46	23	29 2	1 7	− 4
00781	1 893	1 02	56	28	31 3	3 1	−3 0
00391	1 592	1 58	88	44	32 4	4 7	−6 6
00195	1 290	2 24	1 23	62	34 8	7 3	11 6
000977	990	2 93	1 61	81	37 4	13 3	−20 2
000493	693	3 51	1 95	98	38 6	14 3	22 4
000247	393	3 30	1 85	93			

TESTS WITH SPRAYS CONTAINING DISTILLED WATER AND WETTING AGENT

In the second series of tests the spray mixture contained no sodium bicarbonate, and was simply an aqueous solution of a wetting agent (No 2) These experiments were similar to those of the first series except that a different method was used for determining the volume of spray solution retained by the apple The method used here was to remove mechanically and collect the retained liquid by quickly absorbing it into a tuft of absorbent cotton, the cotton being weighed in a glass-stoppered container before and after adsorption of the liquid

These results are presented in table 2 and figure 1, together with values of the relative retention, as explained above

TABLE 2 —*Variation of wetting properties and initial retention with the concentration of wetter No 2 in distilled water*

Concentration of wetter		Spray retained	Relative retention	Surface tension	Interfacial tension	Spreading coefficient
Percent by weight	*Log of grams per cubic meter*	*Cubic centimeters*		*Dynes per centimeter*	*Dynes per centimeter*	*Dynes per centimeter*
1 000	4 000	0 62	0 24	33 8	1 8	−5 1
500	3 699	53	21	32 9	1 3	4 7
200	3 301	80	31	33 6	1 8	−4 9
100	3 000	1 28	50	35 7	4 9	−10 1
0500	2 699	1 74	68	39 7	8 1	−17 3
0200	2 301	1 99	78	39 9	12 6	−22 0
0100	2 000	2 61	1 02	42 3	18 6	−30 4
0000		2 55	1 00	72 0	42 8	−84 1

DISCUSSION

The curves presented in figure 1 indicate that there is a close relationship between the spreading coefficient and the relative retention. The curve for series 1 shows that a relative retention of 0.50 is obtained when the concentration of wetter is 35 gm. (antilog of 1.54) per cubic meter. The curve for series 2 shows the same relative retention at a concentration of 1,050 gm. (antilog of 3.02) per cubic meter. Thus, for the same relative retention the concentration of wetting agent is 30 times as great in series 2 as in series 1. At the same concentrations the corresponding spreading coefficients are −8 and −9. These values agree well, considering the nature of the experiments and the time interval separating the two series.

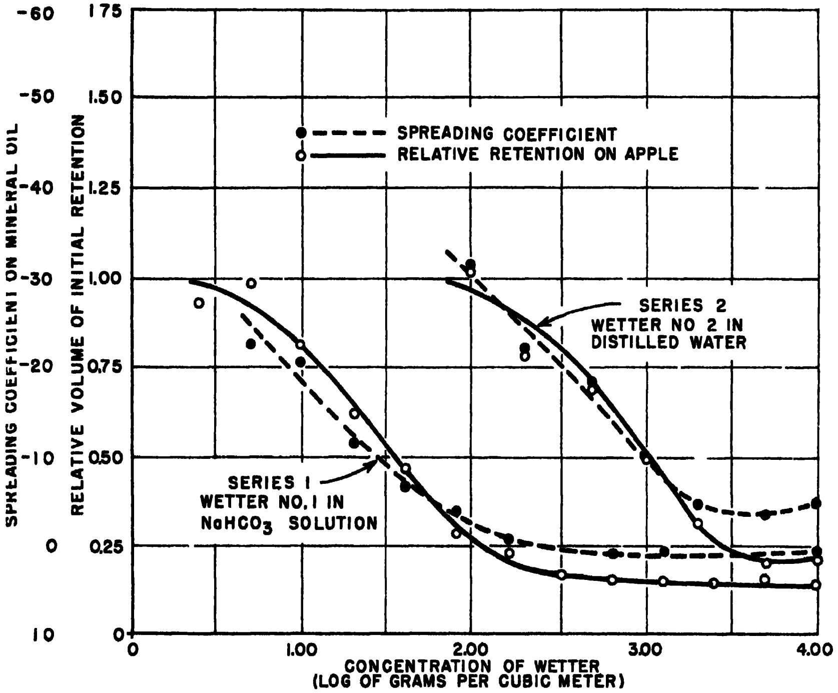

FIGURE 1.—Wetting properties as related to the concentration of wetting agents in spray solutions.

In figures 2 the values of relative retention are plotted against the values of spreading coefficient, and a line has been drawn through the points by inspection. The points representing series 1 lie rather consistently higher than the points representing series 2, and this deviation may possibly be significant. The deviation may be due to a difference in the character of the fruit surfaces used in the two series, or it may be simply a manifestation of the fact that the spreading coefficient is not a perfect measure of wetting power. However, all the points considered together show a good correlation between relative retention and spreading coefficient; i. e., the lower the spreading

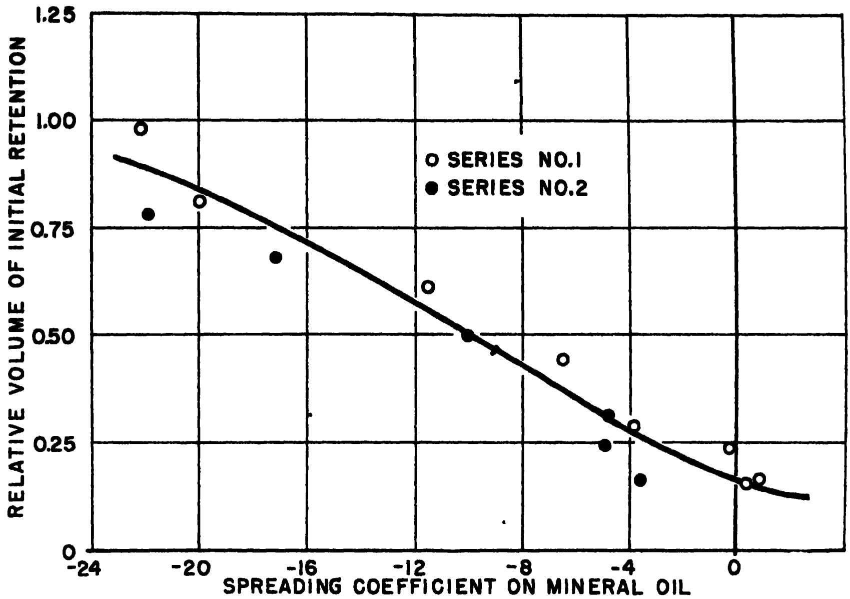

FIGURE 2. Spreading coefficient as related to the initial retention of spray solutions.

coefficient the greater the volume of spray retained. A similar correlation may be expected to occur in the case of other fruit and foliage surfaces. It is suggested that the spreading coefficient on a reference mineral oil may be used as a practical measure of the wetting properties of aqueous spray solutions.

SUMMARY

The relation between the wetting power of a spray mixture and its retention on the sprayed surface has been investigated by spraying a rotating apple with spray solutions of varying wetting power, as measured by their spreading coefficients on mineral oil, and determining the retention of spray mixture at the point of run-off. Plots of the spreading coefficients against the corresponding retentions of spray mixture show that there is a close relationship between these values.

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FACTORS AFFECTING THE GERMINATION OF SUGAR-BEET AND OTHER SEEDS, WITH SPECIAL REFERENCE TO THE TOXIC EFFECTS OF AMMONIA[1]

By MYRON STOUT, *assistant physiologist*, and BION TOLMAN, *assistant agronomist, Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture*[2]

INTRODUCTION

The presence in sugar-beet seed balls of water-soluble substances that produce a toxic effect on germinating seed has been shown in a previous publication (*25*).[3] In the present report the nature of the toxic action is analyzed and the effects of osmotic pressure and of hydrogen-ion concentration are evaluated. Evidence is presented that free ammonia, released during germination from nitrogenous compounds in the water extracts of the seed balls is an important and significant factor in germination inhibition and that this free ammonia produces toxic effects when sugar-beet seed is germinated in the presence of the seed-ball extracts.

REVIEW OF LITERATURE

Extensive work has been reported on the factors affecting the germination of seeds of fleshy fruits when placed on a substratum moistened with the fruit juice. According to Reinhard (*18*), Molisch was the first to express the opinion that the retarded germination was caused by inhibiting substances. The opinion was later supported by Oppenheimer (*14*, *15*), Fukaki (*6*), and Reinhard (*18*). Oppenheimer suggested that the inhibitory substances are colloidal and that they act as "negative catalysts" influencing respiratory processes. Köckemann (*9*) described inhibitory substances, found in various fleshy fruits, that were soluble in water and in ether and were heat-resistant but were destroyed by peroxide and alkali. Reinhard (*18*), working with tomato juice, found inhibiting substances that were water-soluble and heat-resistant but were not destroyed by neutralization.

Osmotic pressure of fruit juices has been considered by some workers a factor in the inhibition of germination. Oppenheimer (*14*, *15*) and Fukaki (*6*) stated that the inhibitory action of substances in tomatoes is something in addition to osmotic effect because boiling the juice increased the osmotic pressure while it reduced the inhibitory action. Recently, Litvinov (*12*) stated that the essential cause of the inhibiting effects of fruit juices is osmotic pressure and that only slight inhibition is traceable to specific organic substances. This conclusion is similar

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to that of Lavialle (*10*), who stated that seeds in all fleshy fruits remain dormant because of the osmotic pressure of their juices, which may vary from 5 atmospheres in tomatoes to 20 atmospheres in grapes.

Other observations pertinent to the studies here reported may be noted. Axentjev (*2*) reported that the inhibiting action of seed extracts is not dependent upon fluorescence. He stated that the action of *Phacelia* seed extract was not specific but that it inhibited the germination of some seeds and stimulated that of others. Mosheov (*13*) reported the presence in wheat of germination-inhibiting substances that were heat-labile and were most effective in light. Lehmann (*11*) found substances having similar properties in the hulls of buckwheat. On the basis of tests with the seeds of *Vaccaria pyramidata*, Borriss (*3*) concluded that the increased germination of seeds planted in soil was not due to any stimulative effect of the soil but rather to the removal of inhibitory substances from the seed by adsorption. Shuck (*20, 21*) reported inhibitory substances, produced by germinating lettuce seed, that were water-soluble and probably a metabolic byproduct. Borthwick and Robbins (*4*) pointed out the possibility that there are deleterious products of metabolism in the endosperm or embryo of lettuce seed, arising and possibly accumulating only at higher temperatures, and that, when other parts are removed, the naked lettuce embryo germinates at these higher temperatures. Thompson (*22*) and Thompson and Kosar (*23*) found soaking and drying, both at low temperatures, and soaking in certain chemical solutions to be effective in breaking the dormancy of lettuce seeds.

MATERIALS AND METHODS

The sugar-beet (*Beta vulgaris* L.) seed balls used in these studies as sources of extracts were principally of two varieties, 68 and U. S. 12. Only one seed lot of variety 68 was available. Previous studies had shown this seed lot to be a heavy carrier of substances that resulted in a toxic effect on germination. The following four lots of seed balls of U. S. 12 were used; these were grown under widely different conditions and varied greatly in chemical composition.

U. S. 12-A.—Normal soil, Hurricane, Utah, 1936 crop. This seed lot was very low in material toxic to germinating seeds.

U. S. 12 B.—Alkali soil, St. George, Utah, 1938 crop. Grown on soil showing considerable white alkali but with fairly good plant survival.

U. S. 12-C.—Extreme alkali soil, St. George, Utah, 1938 crop. Grown on the same field as U. S. 12-B, but in areas where, owing to high alkali content, only a few plants survived and produced seed.

U. S. 12-D.—Normal soil, Moab, Utah, 1938 crop. Owing to insect damage or some other environmental influence, most plants producing this lot of seed reverted to vegetative type of growth during the period when seed normally sets and matures. Viability of the seed was very poor.

Two samples of lettuce seed of the Grand Rapids variety were obtained from T. W. Whitaker.[4] Other kinds of seed such as tomato, radish, cantaloup, cucumber, etc., were obtained from a commercial seed store in Salt Lake City, Utah. These are listed in the various tables.

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[4] Geneticist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, stationed at La Jolla, Calif.

The relative toxicity or germination-inhibiting properties of the extracts or solutions tested were measured by their ability to decrease germination percentages under standardized conditions.

A distinction is sometimes necessary between toxicity and inhibition of germination because certain factors, such as high osmotic pressure, may inhibit germination without resulting in a toxic effect.

The term "toxic substances" as used throughout this report is not limited in meaning to actual substances that are toxic to germinating seeds or plants, but also refers to substances that may not be toxic themselves but from which the toxic agent is produced during the course of seed germination. For example, dilute solutions of asparagin allowed germination to proceed normally for about 48 to 72 hours before sufficient ammonia was released to stop further growth and actually kill the plants.

The seed-ball extracts were prepared by soaking quantities of air-dry seed balls in five times their weight of distilled water for 20 hours, and filtering. Toxicity was determined by germinating naked sugar-beet seeds on thin cotton substrata in 3½-inch Petri dishes moistened with 9 ml. of the solution being tested. Variety 68 was the source of all naked sugar-beet seeds used in the tests.

Osmotic pressure measurements were made by the cryoscopic method, using an air-jacketed sample tube and Beckmann differential thermometer. A glass-electrode pH meter was used in making all pH measurements. Where the pH of extracts or solutions from germination dishes was determined, the sample was obtained by folding the cotton substratum on which the seeds were placed and pressing out the excess solution.

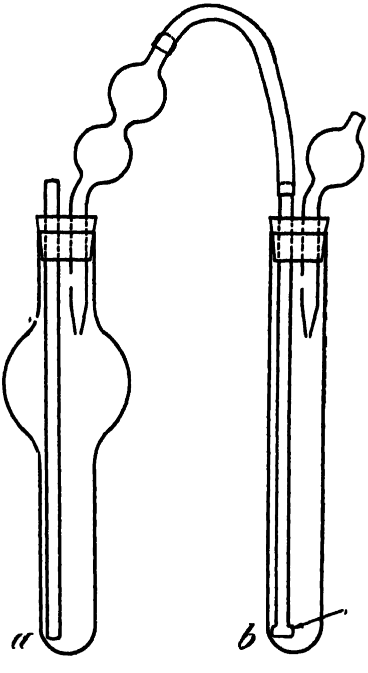

Figure 1.—One unit of ammonia aspiration apparatus. *a*, Aspiration tube; bulb blown for larger samples and to help reduce foaming. *b*, Absorption tube; sintered glass disk in delivery tube and two drops of *n*-butyl alcohol used in solution to insure complete absorption of ammonia.

The nitrogen fractions (free ammonia,[5] combined ammonium salts, and amide nitrogen) were determined by aspirating into boric acid and measuring the conductivity of the solution as outlined by Hendricks et al. (*7*). The aspiration and absorption apparatus was patterned somewhat after that outlined by Sessions and Shive (*19*). One unit is shown in figure 1. A bank of 12 units with accessory equipment is shown in figure 2.

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[5] The term "free ammonia" as used in the present report refers to that ammonia which can be aspirated from a solution without addition of alkaline reagent. Combined ammonium nitrogen refers to the additional ammonia released from its salts by a suitable alkaline reagent. This distinction was made in the present instance even though "free ammonia" has sometimes been used in the literature to indicate ammonia derived from the decompositions of all ammonium salts. The authors consider the latter usage clearly a misnomer.

The *n*-butyl alcohol and sintered glass disks in delivery tubes insured complete absorption, while the warm-water bath and reduced pressure very greatly shortened the time necessary for complete removal of ammonia from the sample. No appreciable phosphate was present in the samples; therefore, magnesium oxide was used as the alkaline reagent. This was done to keep the volume of solution to a minimum for amide nitrogen determination.

The procedure was as follows: 15 ml. of 0.4 molar boric acid solution and 2 drops of *n*-butyl alcohol were added to each absorption tube, and the stopper was pressed firmly in place; 10 ml. of the solution to be analyzed and a drop of mineral oil (to prevent foaming) were added to the sample tubes, the rubber tubes were connected, and aspiration was started. All analyses were run in duplicate or triplicate. Duplicates usually agreed within 0.02 mg. of nitrogen. After aspiration the tubes were disconnected, starting from the end opposite to that at which suction was applied. Suction was decreased gradually as the tubes were disconnected. The contents of the absorption tube was made up to 100 ml. thoroughly mixed, and the conductivity determined. The nitrogen value was read from graphs prepared from conductivity values obtained by adding a known quantity of ammonia to 15 ml. of boric acid and sufficient distilled water to make 100 ml. The plotted curves were nearly straight lines. Combined ammonium nitrogen was determined by adding a small excess of dry magnesium oxide (MgO) to the sample tube and aspirating into fresh boric acid. The acid hydrolysis with 10-percent sulfuric acid, described by Orcutt and Wilson (*16*), was employed for amide nitrogen. After hydrolysis the samples were rinsed back into the original sample tubes and a predetermined amount of strong sodium hydroxide (NaOH), sufficient to nearly neutralize the acid, was added down the side of the tube by means of a pipette. A drop of mineral oil, a drop of phenolphthalein indicator, and an excess of magnesium oxide was added, the flask was immediately closed, and the aspiration was started. Residual nitrogen was determined on the same sample by the Gunning method for organic and ammoniacal nitrogen, with 0.04 N acid and base.

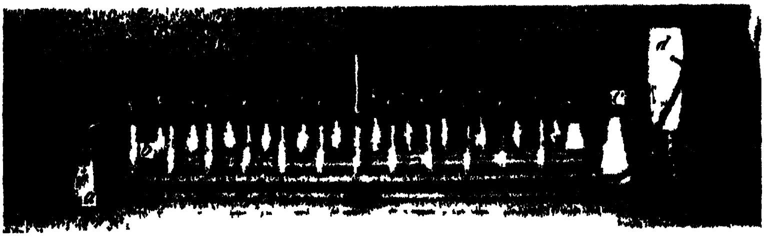

FIGURE 2.—Twelve units of the ammonia aspiration apparatus with accessory equipment. *a*, Suction trap with Bunsen valve. *b*, Hot-water bath for aspiration tubes made from 3-inch galvanized-iron pipe, slightly flattened on sides; vented outlet bent down inside to remove water from the bottom of bath. *c*, Air-scrubber bottles; stopcock for adjusting air intake when operating under reduced pressure. *d*, Hot-water heating coil. Small constant-level feed tank not shown. Note depth of fine bubbles in absorption tubes.

EXPERIMENTAL WORK AND RESULTS

SOME CHEMICAL AND PHYSICAL PROPERTIES OF SUGAR-BEET SEED-BALL EXTRACTS

Water-soluble extracts from sugar-beet seed balls of different varieties and from different seed lots of the same variety varied rather widely in color. The color of fresh extracts usually ranged from amber to dark brown.

All extracts became darker on exposure to the air and darkened rapidly when alkali was added. Normal color was restored upon the addition of excess acid. The extracts turned very dark when ferric chloride was added to them, and a white precipitate was formed with stannous chloride, gelatin, or lead acetate. A voluminous precipitate of gums and pectins was obtained when 95-percent alcohol was added to the concentrated extract. The extracts readily reduced potassium permanganate. Many of the above tests indicated the presence of tannins and gums. Germination tests, however, on pure solutions of tannic acid, gum arabic, etc., indicated that the amounts present could not be responsible for any appreciable degree of toxicity.

Determinations of the heavy metals by means of the dropping mercury electrode showed copper, lead, and zinc to be too low to cause injury.

The fact that the toxicity of the water-soluble extract is due to heat-stable, colloidal, organic matter was indicated by the following tests: The toxicity of the water-soluble extract was reduced when the extract was filtered through one-half of an inch of diatomaceous earth, and it was still further reduced when carbon black was used with the diatomaceous earth. The toxic nature of the extract was not affected by high temperature. Table 1 shows a comparison of the toxic effect of untreated seed-ball extract with that of an extract which had been steamed for 30 minutes at 20 pounds' pressure. When the extract was evaporated to dryness and the residue redissolved in distilled water, little or no change in the effect on germinating seeds was evident. Some of the substances that retarded germination remained in the water-soluble ash, although the effect was not characterized by darkening and killing of the sprouts, as in tests with the normal extract. That retardation in this case could be accounted for by osmotic pressure alone was shown by subsequent tests.

TABLE 1. *Toxicity of heated and normal extracts from sugar-beet seed balls as indicated by their power to inhibit the germination of naked sugar-beet seeds*

[Average of 4 varieties]

Treatment of extract	Naked seeds germinated after		
	19 hours	42 hours	90 hours
	Percent	*Percent*	*Percent*
Steamed 30 minutes at 20 pounds' pressure	17.67	39.83	60.70
Untreated	18.33	15.50	50.00

The solubility of the toxic materials of the seed-ball extracts in various solvents was determined by evaporating a known volume of original extract to near dryness, extracting with a solvent, removing the solvent by successive evaporations with water, redissolving the residue in distilled water to the original volume, and germinating seeds on the various fractions. The toxic materials were found to be soluble in water, or in 80-percent alcohol. They were slightly soluble in absolute alcohol and practically insoluble in propyl alcohol, ether, acetone, or toluene.

Some of the chemical and physical properties of typical sugar-beet seed-ball extracts are shown in table 2. The close correlation between ash, specific conductance, and osmotic pressure indicates that while the total amount of inorganic salts in the extracts varies the relative proportions of the constituent salts remain nearly the same. None of the indices of inorganic salts correlates well with toxicity.

TABLE 2.—*Some properties of sugar-beet seed-ball extracts as related to toxicity*

Source of extract	Osmotic pressure	Total solids	Ash	Specific conductance $\times 10^{5}$ [1]	Total nitrogen	Surface tension [2] per centimeter	Naked seeds germinated after 121 hours
	Atmospheres	*Percent*	*Percent*		*Percent*	*Dynes*	*Percent*
Variety 68 (normal extract)	8.48	1.97	1.087	170.73	0.0045	71.4	3
Variety 68 (filtered through kieselguhr and carbon black)	6.08	1.73	.992	119.54	0.082		12
U. S. 12 A	6.08	1.35	.737	83.05	0.033		70
U. S. 12 B	9.62	2.28	1.201	136.72	.0672	71.1	31
U. S. 12 C	12.27	2.15	1.576	171.91	.1329	[illegible]	22
U. S. 12 D	1.28	1.37	.766	62.10	.0884		21

[1] Reciprocal ohms per centimeter$\times 10^{5}$, 5 ml. diluted to 100 ml. for conductivity measurement.
[2] Surface tension of distilled water at 25° C. = 71.97 dynes per centimeter.

OSMOTIC PRESSURE

Two different types of solutes, sucrose and sodium chloride, were used to determine the tolerance of naked sugar-beet seeds to osmotic pressure. This was done to check any effects that might be due to factors other than osmotic pressure. Table 3 shows that sugar-beet seeds are tolerant to fairly high osmotic pressure and that the two solutions used gave fairly similar results.

TABLE 3.—*Influence of osmotic pressure on the germination of naked sugar-beet seeds*

Solute used	Osmotic pressure	Naked seeds germinated after 25 hours	49 hours	73 hours	142 hours
	Atmospheres	*Percent*	*Percent*	*Percent*	*Percent*
Water (check)	0	64	88	99	99
Sucrose	2.12	67	85	96	99
	4.29	67	89	93	97
	6.12	55	69	79	85
	8.40	46	67	77	82
	12.55	24	40	62	60
	18.53	4	7	14	26
	23.67	0	0	3	13
Sodium chloride	2.00	75	88	94	96
	3.94	60	74	85	87
	5.78	46	69	78	84
	7.73	38	68	67	74
	11.28	15	35	50	57
	15.55	8	21	34	35
	19.54	0	4	12	13

Several other solutes were used in concentrations having osmotic pressure values similar to that of variety 68 sugar-beet seed-ball extract. The data in table 4 show that these isotonic chemical solutions varied in their influence on seed germination. Differences in germination up to 70 percent were observed on solutions having similar osmotic pressure. Urea, for example, was outstanding in toxicity. The tips of the radicles from the seeds that germinated in the presence of urea were similar in appearance to those of radicles from seeds germinated on sugar-beet seed-ball extracts.

It is evident from the data that important toxic effects on germinating seed cannot be explained on the basis of osmotic pressure.

TABLE 4.—*Influence of sugar-beet seed-ball extracts and of chemical solutions isotonic with certain seed-ball extracts on the germination of naked sugar-beet seeds*

Extract or solution used to moisten substratum	Osmotic pressure	Naked seeds germinated after—		
		22 hours	46 hours	121 hours
Sugar beet variety	*Atmospheres*	*Percent*	*Percent*	*Percent*
68 (extract)	8.48	0	3	3
68 (extract diluted with 2 parts water)	3.36	9	9	25
68 (extract filtered through kieselguhr and carbon black)	6.08	0	4	12
Urea	8.25	1	2	3
Ammonium sulfate	8.48	13	33	39
Sodium nitrate	8.57	13	29	39
Sodium chloride	8.48	17	45	57
Sucrose	8.37	25	63	73
Water (check)	0	54	92	94

TOXICITY OF AMMONIA

RELEASE OF AMMONIA DURING GERMINATION

The known instability of urea and a faint odor from the urea, similar to that from seed-ball extracts, suggested that ammonia might be released during germination.

Germination tests were conducted with naked sugar-beet seeds on seed-ball extracts and chemical solutions hermetically sealed in glass dishes. After 71 hours 2 liters of ammonia-free air was passed through the dishes and Nessler's solution. The results of this test showed that ammonia was liberated from all the seed-ball extracts and solutions containing nitrogen and that there was an increase in alkalinity during germination (table 5). A comparison of the effect of the dilute ammonia and urea solutions (table 5) shows that the concentration of ammonia was greatest at the beginning of the test in the dishes containing the ammonia solution; germination was completely inhibited, and alkalinity was high but decreased during the test. The dishes containing urea solutions started with a comparatively low pH and low initial concentration of ammonia, which allowed some germination to start. During the test, however, the ammonia and alkalinity both increased to the point where more ammonia was in equilibrium with the air in the dishes containing urea than in those containing the ammonia solution. The behavior of asparagin solutions in this test was also interesting. Evidently very little ammonia was released until after about 2 days, when some apparently normal germination had taken place. Soon after this, however, the seedlings turned brown and no further germination occurred. This delayed action was evident in all subsequent tests with asparagin.

TABLE 5.—*Free ammonia content of air drawn through vessels containing naked sugar-beet seeds germinating on various extracts and solutions*

Extract or solution	Osmotic pressure	pH of fresh solution	Results after 71 hours		
			pH	Naked seeds germinated	Nitrogen[1]
Sugar-beet variety	*Atmospheres*			*Percent*	*Mg*
08 (extract)	8.48	6.27	8.20	9	0.027
U. S. 12-A (extract)	6.08	5.50	7.65	29	.009
U. S. 12-B (extract)	9.62	5.20	7.80	20	.010
U. S. 12-C (extract)	12.27	4.75	7.51	16	.010
U. S. 12-D (extract)	4.28	5.50	8.16	21	.015
Ammonia (0.4 mg. N per ml.)		10.50	8.16	0	.050
Urea (1.9 percent)	8.25	7.00	8.55	3	.080
Urea+1 percent NaCl[2]	8.30	5.13	8.06	15	.040
Asparagin (4.2 percent)			8.00	31	.060
Asparagin+1 percent NaCl[2]	8.30	4.33	8.10	40	.030
Sodium nitrate	8.57	5.75	7.72	32	.009
Ammonium sulfate	8.48	5.20	6.48	35	.005
Water (check)			6.20	96	0

[1] As ammonia in 2 liters of air.
[2] Contained approximately 1 mg. of nitrogen per milliliter.

Further evidence on the relation of the rapidity of ammonia release from nitrogenous compounds to toxicity was obtained in an experiment with seed-ball extracts. In this test the ammonia from the most readily broken down nitrogen compounds was removed from the seed-ball extract, and the toxicity of the modified extract was then compared with a portion of the same extract from which practically no ammonia had been removed. Two hundred milliliters of heated seed-ball extract was divided and 25 ml. of an extract from germinating seeds was added to one of the 100-ml. portions (*A*). An equal amount of heated extract from germinating seeds was added to the

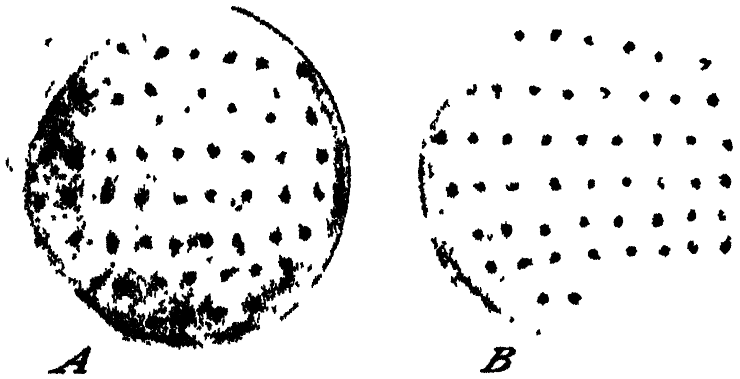

FIGURE 3. Relation of the nitrogen content of seed-ball extracts to delayed toxicity: *A*, Seed-ball extract from which 0.272 mg. of nitrogen as ammonia per milliliter was removed prior to testing toxicity; *B*, seed-ball extract from which practically no nitrogen had been removed prior to testing toxicity. Note almost complete inhibition of germination in *B* and delayed toxicity in *A*; darkening of sprouts in *A* is typical of ammonia injury.

other portion (*B*) of seed-ball extract, and both mixtures were incubated for 4 days. Both samples were then aspirated; 0.272 mg. of nitrogen as ammonia per milliliter of solution was removed from *A*, while only 0.002 mg. was removed from *B*. Both samples were then heated to stop further action, and their toxicity to germinating seeds compared. Although the final pH of both lots after 90 hours of germination was practically the same and sprouts from both samples were ultimately killed, germination counts showed that the removal of more ammonia from *A* resulted in increased germination (fig. 3, *A* and *B*). Forty-eight percent of the seed on the seed-ball extract from which very little ammonia had been removed germinated in 90 hours, while 81 percent of the seed on the seed-ball extract from which the larger amount of ammonia had been removed germinated.

TOXIC EFFECT OF AMMONIA ON SUGAR-BEET SEEDS AND SEEDLINGS

To obtain further information on the effect of ammonia, tests were conducted to determine the tolerance of seedlings and older sugar-beet plants to ammonia solutions and sugar-beet seed-ball extracts. Three-week and two-month-old plants were supported through corks and their roots submerged in seed-ball extracts and in an ammonia solution containing 0.4 mg. of nitrogen as ammonia per milliliter. All of the smaller plants collapsed within 4 hours. The roots of the larger plants turned black, and the leaves began to dry in 18 hours. Water checks started at the same time were still living at the end of 2 months.

In another test small seedlings were placed in water culture and the vials put in a larger container. Enough of the above-mentioned ammonia solution was placed in the large container to cover the bottom to a depth of one-quarter of an inch, and the large container was then sealed. Although the small seedlings were not in contact with the ammonia solution, within 2 hours sufficient ammonia had accumulated within the aerial parts to cause their collapse. Subsequent tests, reported in table 6, showed that ammonia concentrations above 0.1 mg. of nitrogen per milliliter decreased germination of naked sugar-beet seeds. Further tests (see fig. 4) indicated that much lower concentrations of ammonia, if effectively maintained, would inhibit germination.

TABLE 6. *Influence of several different concentrations of free ammonia on germination of naked sugar-beet seeds*

Nitrogen[1] (milligrams)	pH of fresh solution	pH of solution after germination	Naked seeds germinated after 22 hours	Naked seeds germinated after 65 hours	Naked seeds germinated after 120 hours
			Percent	*Percent*	*Percent*
Water (check)	5.57	6.20	53	95	95
0.025	8.50	6.51	47	96	96
.050	9.35	7.01	39	93	93
.100	9.90	7.51	21	69	74
.200	10.20	8.12	12	56	57
.300	10.30	8.30	7	12	11
.400	10.50	8.40	0	0	0
.800	10.72	8.53	0	0	0

[1] As ammonia per milliliter of solution.

NITROGEN ANALYSIS OF SUGAR-BEET SEED-BALL EXTRACTS AND CHEMICAL SOLUTIONS BEFORE AND AFTER GERMINATION

To determine whether toxic amounts of ammonia were released from certain chemical solutions and seed-ball extracts by germinating seed, nitrogen analyses were made on seed-ball extracts and chemical solutions immediately after their preparation and also after their recovery from the cotton substratum on which naked sugar-beet seeds had been germinated for 5 days. The results of these analyses are shown in table 7. Free ammonia was liberated from the nitrogen compounds of all the extracts and synthetic solutions. The presence of the liberated ammonia is reflected in the rise in pH. The pH values after germination (table 7) did not rise so high during germination as in several other instances where germination was conducted in smaller dishes and on thinner substrata. The significant data in table 7 are the increases in the amount of free ammonia in the substrata after seed germination. The amounts in most instances are more than sufficient to cause inhibition of germination, especially since the ammonia was released during germination and tended to increase as germination proceeded rather than to be decreased by loss or neutralization, as was evident in tests where ammonia solutions were used. The amounts of combined ammonium ion serve to indicate the relative buffering capacities of the solutions or extracts. No significance was attached to the small changes in amide nitrogen. Residual and total nitrogen determinations were merely checks on technique.

TABLE 7.—*Analyses of the nitrogenous constituents of sugar-beet seed-ball extracts and solutions of urea and asparagin*

[Analyses of fresh solutions and of solutions recovered from substrata on which seeds had been germinated]

Source of extract or synthetic solution	Fresh solution						Solution after germination (5 days)					
	pH	Nitrogen [1] as—					pH	Nitrogen [1] as—				
		Free NH_3	Combined NH_4^+	Amide N	Residual N	Total N		Free NH_3	Combined NH_4^+	Amide N	Residual N	Total N
Sugar-beet variety:		*Mg*	*Mg*	*Mg*	*Mg*	*Mg*		*Mg*	*Mg*	*Mg*	*Mg*	*Mg*
68	6.27	0	0.081	0.199	0.398	0.678	7.70	0.315	0.044	0.159	0.157	0.675
U. S. 12-A	5.50	0	.049	.066	.368	.483	7.58	.228	.067	.034	.201	.530
U. S. 12-C	4.75	0	.100	.132	1.097	1.329	7.64	.364	.216	.187	.575	1.342
U. S. 12-D	5.50	0	.073	.163	.666	.902	7.55	.370	.020	.066	.361	.817
Urea+1 percent NaCl	5.13	0	.001	.198	.799	.998	7.58	.146	.034	.325	.534	1.039
Asparagin+1 percent NaCl	4.33	0	.000	.386	.437	.823	7.58	.707	.085	.012	.102	.906

[1] Measured as milligrams per milliliter of solution

AMMONIA RELEASED BY ENZYMATIC HYDROLYSIS

Further studies were conducted to determine the source of the hydrolytic agent responsible for the release of free ammonia from seed-ball extracts and certain chemical solutions containing organic nitrogen. Two sets of Petri dishes were prepared for germination tests. The substrata of one set were moistened with sugar-beet seed-ball extract and those of the other with 0.25-percent urea solution. The dishes were covered and steamed at 20 pounds' pressure for 30 minutes and were then cooled, and naked sugar-beet seeds that had been shaken with toluene were placed in one-half of each lot of dishes. All planting was done under a General Electric germicidal lamp (2,537 A. U. radiation) in a closed inoculation chamber. After 93 hours the liquid was recovered from the dishes and immediately analyzed. The result of the analysis (table 8) indicates that the seed carries a hydrolytic agent that is probably enzymatic in character.

Tests were run in which germinating seeds were ground and extracted with water. This extract was added to sugar-beet seed-ball extract and chemical solutions, and analyzed immediately after mixing and after periods of incubation at 30° C. One flask containing seed-ball extract and the above-mentioned extract from germinating seed was heated at 20 pounds' pressure for 30 minutes. The analyses are presented in table 9. It is evident from the data in table 9 that an extract prepared from germinating beet seed has hydrolytic properties similar to those of intact germinating seeds and that this hydrolytic agent is heat-labile.

TABLE 8.—*Nitrogen released by germinating sugar-beet seeds under sterile conditions*

[Seeds were washed in toluene]

Treatment	pH	Nitrogen as free ammonia per milliliter
		Milligrams
Sugar beet seed-ball extract (no seeds)	5.79	0.007
Sugar beet seed-ball extract (50 seeds per dish)	8.23	.251
0.25 percent urea (no seeds)	7.63	.032
0.25 percent urea (50 seeds per dish)	8.61	.443

TABLE 9.—*Analyses of seed-ball extracts and of chemical solutions incubated in glass bottles at 30° C. with a solution prepared from germinating naked sugar-beet seeds*

Source of extract or solution	Incubation period	pH	Nitrogen per milliliter of solution: Free NH_3	Combined NH_4^+	Amide N	Residual N	Total N
Sugar beet variety	*Days*		*Mg*	*Mg*	*Mg*	*Mg*	*Mg*
U. S. 12-D	0	5.50	0	0.132	0.070	0.542	0.744
	3		.189	.073	.044	.450	.756
	6	7.30	.170	.087	.081	.436	.774
U. S. 12-D (steamed at 20 pounds' pressure for 30 minutes)	2	5.50	.002	.170	.054	.584	.810
	4		.002	.179	.065	.599	.845
	6	5.57	.001	.158	.089	.589	.837
68	0	6.27	0	.119	.171	.355	.645
	3		.301	.023	.053	.269	.646
	6	8.23	.305	.036	.043	.258	.642
Asparagin+1 percent NaCl	0	4.33	0	.019	.317	.378	.714
	3		.324	.318	.010	.074	.726
	6	7.50	.350	.290	.015	.080	.735
Urea+1 percent NaCl	0	5.13	0	.008	.176	.637	.821
	3		.217	.064	.077	.459	.817
	6	8.70	.325	.054	.124	.341	.844

The previous tests indicated the presence of urease, asparaginase, and probably other proteases. The presence of urease in naked sugar-beet seeds was shown by employing the technique described by Annett (*1*). Five grams of naked sugar-beet seeds was ground to a fine powder and shaken with 50 ml. of distilled water in the presence of toluene for 1½ hours. The mixture was filtered, and 10 ml. of the filtrate was added to 50 ml. of 0.25-percent urea solution. Some of the samples received 0.5 ml. of toluene each; no toluene was added to the others. Five ml. of the mixtures was titrated immediately with 0.035 N hydrochloric acid (HCl) and like amounts at various intervals thereafter over a period of 165 hours. Methyl red was used as the indicator. The results are shown in table 10. It is evident from the data in table 10 that the urease content of sugar-beet seed is very low as compared to that of soybeans, swordbeans, or jackbeans, and that the hydrolysis is decreased by the addition of toluene. The decreased hydrolysis in the presence of toluene may have been due to decreased activity of urease or to slight microbial action in the absence of toluene.

TABLE 10 *Urease content of naked sugar-beet seeds*[1] *as indicated by the quantities of hydrochloric acid (0.035 N) used in titrations*

Time elapsed from beginning of test (hours)	Hydrochloric acid required with—		
	Urea (toluene check)	Urea + enzyme	Urea + enzyme + toluene
	Milliliters	*Milliliters*	*Milliliters*
	0	0.10	0.10
	0	.42	.40
21	0	1.00	.90
45	0	1.60	1.18
69	0	2.08	1.92
93	0	2.95	2.25
117	0	3.50	2.40
165	0	4.20	2.58
	pH	*pH*	*pH*
Reaction at 165 hours	6.70	8.73	9.00

[1] 5 ml of sample titrated with 0.035 N hydrochloric acid, methyl red used as indicator

pH STUDIES

The ammonia toxicity previously encountered was associated with a rise in pH. Therefore, to evaluate separately the influence of pH and ammonia, further tests were conducted. The changes in the pH of several seed-ball extracts and solutions that were observed during germination tests are shown in table 11. Several attempts were made to stabilize the pH of extracts at low values and the pH of prepared solutions at high values to test the range at which seeds would germinate. Several buffer solutions were tried, none of which successfully stabilized pH during germination. The results of some of the tests are given in table 12. The addition of either citric acid or calcium sulfate to seed-ball extracts retarded the toxic action on germinating seed and permitted greater sprout development. Ultimately, however, the killing of root tips was just as pronounced and the rise in pH nearly as high as when no buffer was used. The effect of germinating seeds on prepared solutions of high initial pH was similar to that already noted on ammonia solutions. The drop in pH of these solutions must have been brought about by the buffering action of organic acids and carbon dioxide liberated during germination, which indicates that the initial stages of germination can proceed when alkalinity is relatively high. Further tests to determine the influence of alkalinity and of ammonium hydroxide on the germination of seeds and growth of sprouts were made by subjecting the thin cotton substrata on which the seeds were placed to a continuous flow of solutions of definite pH values.

TABLE 11.—*pH values of several sugar-beet seed-ball extracts and of chemical solutions before and after naked sugar-beet seeds had been germinated on them for 7 days*

Source of extract or solution	pH of fresh solution	pH of solutions after seeds were germinated on them	Difference in pH before and after germination
Distilled water	[1]5	6.81	1.24
Sugar-beet variety:			
U. S. 12-A	5.50	7.90	2.40
U. S. 12-B	5.20	7.80	2.60
U. S. 12-C	4.75	7.54	2.79
U. S. 12-D	5.50	8.65	3.15
U. S. 12-D (no seeds)[2]	5.50	5.79	.29
C8	6.27	8.63	2.36
Asparagin and salt	7.33	8.35	1.02
Urea and salt	5.13	8.43	3.30
Urea	7.00	8.55	1.55
$NaNO_3$	5.75	7.22	1.47
$(NH_4)_2SO_4$	5.20	6.48	1.28
0.10 mg. N as NH_3 per ml.	10.20	8.10	−2.10
0.20 mg. N as NH_3 per ml.	10.20	8.05	−2.15

[1] Average of several measurements during tests; has no significance except to show trend of change during germination tests.

[2] Check to show pH change without germinating seeds.

TABLE 12.—*Influence of buffers on change in pH of extracts and several solutions during germination of naked sugar-beet seeds*

Source of extract or solution	pH of fresh solution	pH of solution after seed had germinated	Germination period	Naked seeds germinated
Sugar-beet variety:			*Hours*	*Per cent*
U. S. 12 D. Diluted 1:1 with citric acid solution	4.20	5.75	120	86
U. S. 12 D. Diluted 1:1 with water	5.60	5.83	126	70
U. S. 12 D. Diluted 1:1 with calcium sulfate solution	5.00	5.7	91	82
Sodium hydroxide	8.10	6.70	72	90
	8.90	6.90	72	91
	10.70	7.18	72	90
Sodium carbonate	10.60	8.08	72	86
Calcium carbonate	8.30	6.90	72	91
Disodium phosphate plus sodium carbonate plus tartaric acid	9.2	5.67	88	92
	9.68	9.12	88	88
1 percent disodium phosphate plus sodium hydroxide	8.72	7.75	41	50
	9.70	7.80	41	58
	10.12	8.00	41	46
	10.57	8.20	41	52
	11.12	8.57	41	12
	11.0	9.21	41	8

In a test designed to determine the effect of a maintained pH as obtained by a continuous flow of solution through the substratum the following method was used. The seeds were placed on the cotton substratum, after which the Petri dishes were covered and sealed with wax, and a flow of about 100 ml. per hour was allowed to drip through a sealed-in glass tube, on the upper edge of the cotton, then flow through the substratum to the opposite side of the dish, and discharge through a small hole drilled in the bottom of the dish. A small end of the cotton substratum pulled through the hole in the bottom of the dish served as a wick to remove the excess solution without allowing the entrance of air. The results of these tests (tables 13, 14, and 15) show that seeds will germinate at a fairly high pH on such solutions as monopotassium phosphate (KH_2PO_4) and sodium carbonate (Na_2CO_3), where potassium hydroxide (KOH) was used to raise the pH. Some injury was noticed at the higher pH values on sodium carbonate solutions, but this was slight compared to that produced by ammonia at much lower pH values. The relative effect of the three kinds of solution on germination percentage and the increased effectiveness of maintained concentrations are shown in figure 4.

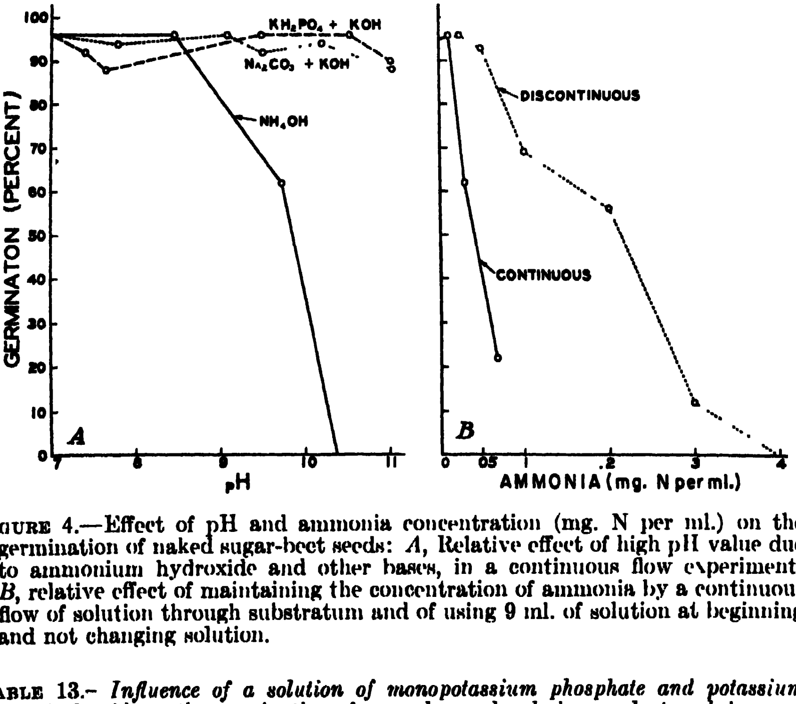

FIGURE 4.—Effect of pH and ammonia concentration (mg. N per ml.) on the germination of naked sugar-beet seeds: *A*, Relative effect of high pH value due to ammonium hydroxide and other bases, in a continuous flow experiment; *B*, relative effect of maintaining the concentration of ammonia by a continuous flow of solution through substratum and of using 9 ml. of solution at beginning and not changing solution.

TABLE 13.—*Influence of a solution of monopotassium phosphate and potassium hydroxide on the germination of cucumber and naked sugar-beet seeds*[1]

Kind of seed	pH of supply solution	20 hours		44 hours	
		Seeds germinated	pH	Seeds germinated	pH
		Percent		*Percent*	
Cucumber seeds	8.30	56	7.37	100	7.18
	9.40	32	7.77	100	6.75
	9.98	44	9.35	92	8.31
	10.68	40	10.52	96	10.12
	11.06	44	10.53	84	11.01
Naked sugar-beet seeds	8.30	54	7.67	88	7.65
	9.40	54	8.18	92	7.40
	9.98	54	9.76	96	9.48
	10.68	51	10.63	96	10.55
	11.06	68	11.03	90	11.01

[1] 1 gm. of KH_2PO_4 per 18 liters of solution; KOH used to raise pH to value noted.

It is also evident from table 15 that the amount of ammonium salt present at a fairly high pH is important. The solution having a pH of 9.31 and containing 0.067 mg. of nitrogen per milliliter showed greater toxicity than the one having a pH of 9.72 but containing only 0.029 mg. of nitrogen per milliliter. The fact that, when the concentration is maintained, a solution containing a very small amount of ammonia may be toxic has apparently not been recognized.

TABLE 14.—*Influence of sodium carbonate solutions at various pH values on the germination of cucumber and naked sugar-beet seeds*

Kind of seed	Na_2CO_3 added to 18 liters of H_2O	pH of supply solution	18 hours: Seeds germinated	18 hours: pH	44 hours: Seeds germinated	44 hours: pH	68 hours: Seeds germinated	68 hours: pH	68 hours: Average length of sprouts
	Grams		*Percent*		*Percent*		*Percent*		*Mm.*
Cucumber seeds	0.3	8.70	84	7.54	92	7.68	96	7.82	17.4
	1.0	9.60	88	8.88	96	9.06	96	9.20	20.4
	2.1	9.96	84	9.35	100	9.56	100	9.57	14.4
	3.0+KOH	10.62	88	9.98	96	10.[illegible]7	9[illegible]	10.25	6.5
	3.0+KOH	11.34	88	10.98	100	10.99	100	10.96	5.5
Naked sugar beet seeds	.3	8.70	66	7.60	94	[illegible].84	94	7.80	6.0
	1.0	9.60	64	8.92	96	9.20	96	9.10	4.6
	2.1	9.96	60	9.44	84	9.48	92	9.52	3.7
	3.0+KOH	10.62	64	10.22	82	10.2[illegible]	91	10.22	3.0
	3.0+KOH	11.34	52	11.03	68	11.0[illegible]	88	11.0[illegible]	2.2

TABLE 15.—*Influence of ammonia solutions at various pH values on the germination of cucumber and naked sugar-beet seeds*

Kind of seed	pH of supply solution	Nitrogen[1]	24 hours: Seeds germinated	24 hours: pH	48 hours: Seeds germinated	48 hours: pH	72 hours: Seeds germinated	72 hours: pH	72 hours: Average length of sprouts
		Milligram	*Percent*		*Percent*		*Percent*		*Millimeters*
Cucumber seeds	8.48	0.014	90	8.05	88	7.91	92	8.07	24.00
	9.31	.067	8	9.09	92	9.29	9[illegible]	9.19	2.44
	9.72	.029	40	9.46	84	9.63	92	9.50	3.48
	10.38	.647	0	10.16	0	10.37	0	10.28	0
Naked sugar beet seeds	8.48	.014	74	8.27	90	8.38	96	8.18	1.00
	9.31	.067	16	9.18	22	9.29	22	9.25	.38
	9.72	.029	28	9.50	52	9.68	62	9.65	1.34
	10.38	.647[2]	0	10.12	0	10.38	0	10.32	0

[1] Measured as milligrams per milliliter of fresh solution.

[2] The pH value of this solution was overstepped; a small amount of phosphoric acid (H_3PO_4) was therefore added and the pH readjusted with NH_4OH.

INFLUENCE OF OSMOTIC PRESSURE AND AMMONIA ON THE GERMINATION OF CERTAIN SEEDS

The toxicity of the water-soluble extract from sugar-beet seed balls is not specific for sugar-beet seed but also affects the germination of several other seeds. Table 16 shows the effect of sugar-beet seed-ball extract on the germination of onion, lettuce, radish, cucumber, cantaloup, tomato, sweet corn, and bean seeds. The germination of some of these seeds was totally inhibited while that of others was merely retarded. There was either an inhibitory or retarding effect on the germination of all kinds of seed on which the extract was tested.

TABLE 16.—*Influence of seed-ball extract of sugar-beet variety 68 on germination of seeds of various plants*

Kind and variety of seed	Treatment	Seeds germinated after 19 hours	45 hours	64 hours	90 hours	138 hours
		Percent	*Percent*	*Percent*	*Percent*	*Percent*
Sugar beet (Variety 68, naked)	Water (check)	56	86	88	88	88
	Extract	0	3	5	5	6
Radish (Scarlet White Tip)	Water (check)	36	60	72	81	90
	Extract	0	2	2	2	2
Lettuce (New York No. 12)	Water (check)	70	84	84	86	88
	Extract	0	0	0	0	0
Cucumber (Klondike White Spine)	Water (check)	66	94	98	98	98
	Extract	0	50	66	[1] 66	66
Onion (Sweet Spanish or Valencia)	Water (check)	0	58	74	78	82
	Extract	0	0	8	10	10
Tomato (New Stone)	Water (check)	0	60	92	96	96
	Extract	0	0	4	4	4
Cantaloup (Hale Best)	Water (check)	0	38	66	72	76
	Extract	0	0	0	0	0
Sweet corn (Golden Bantam)	Water (check)	0	85	100	100	100
	Extract	0	0	40	75	80
Beans (Red Kidney)	Water (check)	0	5	35	55	70
	Extract	0	0	35	55	65

[1] Tips of radicles barely visible; no subsequent growth.

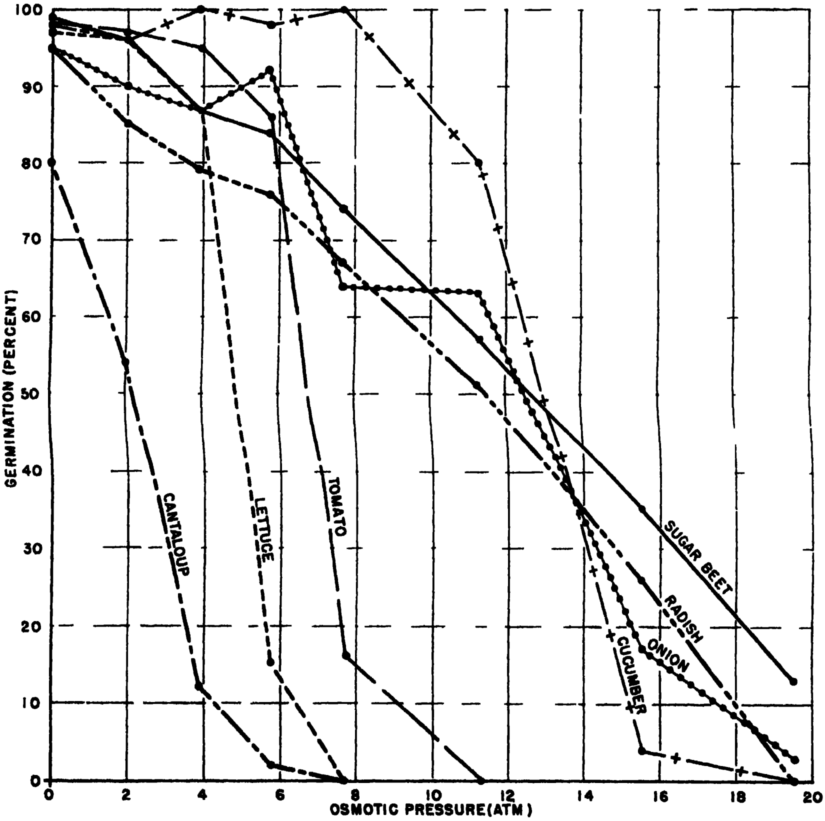

FIGURE 5.—Effect of osmotic pressure on the ultimate germination of several kinds of seeds on a substratum moistened with a sodium chloride solution.

TABLE 17.—*Influence of osmotic pressure on the germination of several kinds of seeds*

[Substratum moistened with water, sucrose, and a sodium chloride solution]

Kind and variety of seed	Solution used	Osmotic pressure	Seeds germinated after— 25 hours	49 hours	73 hours	142 hours	257 hours
		Atmospheres	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Radish (Scarlet White Tip)	Water (check)	----	68	81	88	95	-
	Sucrose	2.12	36	57	72	76	----
		4.29	25	56	61	67	-----
		6.12	23	54	62	67	-------
		8.40	3	47	55	59	---
		12.55	1	12	20	26	---
		18.53	0	1	2	6	---
		23.67	0	0	0	0	-- -
	Sodium chloride	2.00	51	74	83	85	---
		3.94	33	56	70	79	----
		5.78	31	60	66	76	-----
		7.73	20	46	59	67	- -
		11.28	6	17	37	51	---
		15.55	0	4	12	26	- -
		19.54	0	0	0	0	- -
Lettuce (New York No. 12)	Water (check)		90	94	96	97	-----
	Sucrose	2.12	71	81	87	92	- --
		4.29	47	63	72	79	-- --
		6.12	1	16	19	21	--
		8.40	0	0	0	0	- -
	Sodium chloride	2.00	89	91	96	96	- -
		3.91	55	72	78	87	- --
		5.78	3	10	11	15	- -
		7.73	0	0	0	0	--
Cucumber (Klondike White Spine)	Water (check)		46	94	96	98	98
	Sucrose	2.12	30	94	96	98	98
		4.29	4	91	100	100	100
		6.12	0	86	98	100	100
		8.40	0	28	70	84	84
		12.55	0	0	0	6	8
		18.53	0	0	0	0	0
	Sodium chloride	2.00	30	96	96	96	96
		3.94	14	90	100	100	100
		5.78	0	92	96	98	98
		7.73	0	66	96	100	100
		11.28	0	0	36	68	80
		15.55	0	0	0	0	4
		19.54	0	0	0	0	0
Onion (Sweet Spanish, or Valencia).	Water (check)		0	15	69	89	95
	Sucrose	2.12	0	1	51	87	92
		4.29	0	2	19	73	88
		6.12	0	0	7	61	78
		8.40	0	0	2	39	72
		12.55	0	0	0	1	16
		18.53	0	0	0	0	2
		23.67	0	0	0	0	0
	Sodium chloride	2.00	0	0	40	80	90
		3.94	0	1	28	77	87
		5.78	0	0	12	83	92
		7.73	0	0	2	39	64
		11.28	0	0	2	19	63
		15.55	0	0	0	1	17
		19.54	0	0	0	0	3
Tomato (New Stone)	Water (check)		0	1	33	94	98
	Sucrose	2.12	0	0	5	78	97
		4.29	0	0	0	19	74
		6.12	0	1	1	1	19
		8.40	0	0	0	0	0
	Sodium chloride	2.00	0	0	0	35	97
		3.94	0	0	0	8	95
		5.78	0	0	0	0	86
		7.73	0	0	0	0	16
		11.28	0	0	0	0	0
Cantaloup (Hale Best)	Water (check)		0	0	16	50	80
	Sucrose	2.12	0	0	2	4	6
		4.29	0	0	0	0	0
	Sodium chloride	2.00	0	0	1	10	54
		3.94	0	0	0	2	12
		5.78	0	0	0	0	2
		7.73	0	0	0	0	0

After it had been demonstrated that there was some differential response of several different kinds of seeds to sugar-beet seed-ball extracts, further tests were conducted to determine the relative tolerance of these seeds to osmotic pressure. The data in table 17 and figure 5 indicate that there was a differential response to osmotic pressure. It is evident that seeds of sugar beet, onion, radish, and cucumber were not so sensitive to osmotic pressure as those of lettuce, tomato, and cantaloup. Furthermore, there was no typical darkening and killing of the sprouts with sucrose and sodium chloride solutions such as had occurred when sugar-beet seed-ball extract was used.

The tolerance of these same seeds to dilute ammonia solutions is shown in table 18 All seeds tested were retarded in germination by concentrations of from 0.2 to 0.4 mg. of nitrogen as ammonia per milliliter. The pH of the substratum was lowered even more than in tests with beet seeds. Further tests showed that the germination of these seeds was completely inhibited when the concentration of free ammonia was maintained, as in the test on cucumber and naked sugar-beet seeds (table 15 and fig. 4).

TABLE 18 — *Toxicity of ammonia as indicated by its power to inhibit the germination of various seeds*

Kind and variety of seed germinated	N as NH_3 per milliliter of fresh solution	pH of fresh solution	pH after germination	Seeds germinated after— 48 hours	78 hours	120 hours
	Milligram			*Percent*	*Percent*	*Percent*
Radish (Scarlet White Tip)	[1] None	5.57		60	78	86
	0.1	9.90	4.82	64	88	94
	.2	10.20	4.78	34	58	88
	.3	10.30	4.99	6	50	64
	.4	10.50	5.76	2	38	60
Onion (Sweet Spanish or Valencia)	[1] None	5.57		58	76	82
	.1	9.90	4.91	24	82	86
	.2	10.20	5.78	24	76	84
	.3	10.30	6.83	4	56	64
	.4	10.50	8.07	2	10	46
Lettuce (New York No 12)	[1] None	5.57		84	84	86
	.1	9.90	4.81	92	92	92
	.2	10.20	7.50	0	0	28
	.3	10.30	7.73	0	0	0
	.4	10.50	7.83	0	0	0
Tomato (New Stone)	[1] None	5.57		60	94	96
	.1	9.90	5.17	24	78	86
	.2	10.20	5.20	6	86	90
	.3	10.30	6.20	0	66	80
	.4	10.50	6.28	0	22	78
Cantaloup (Hale Best)	[1] None	5.57		58	60	74
	.1	9.90	6.80	0	40	76
	.2	10.20	7.18	0	42	60
	.3	10.30	6.92	0	12	56
	.4	10.50	7.10	0	0	20
Cucumber (Klondike White Spine)	[1] None	5.57	6.10	0	96	100
	.1	9.90	5.95	0	100	100
	.2	10.20	5.72	0	94	98
	.3	10.30	6.65	0	82	96
	.4	10.50	7.31	0	40	98

[1] Water, check solution

SOME PROPERTIES OF EXTRACTS FROM SEVERAL DRY FRUITS

Studies were conducted to determine whether the physical and chemical properties of water-soluble extracts of other dry fruits were similar to those of sugar-beet seed-ball extracts. Extracts were prepared from the ripened seeds of lettuce, oats, barley, spinach, and celery Table 19 shows some of the properties of these extracts and also a comparison of their effect on the germination of both lettuce and naked sugar-beet seeds. It is evident that the extracts from all of the seeds tested had some retarding effect on the germination of both beet and lettuce seeds. With the extracts of barley, oats, spinach, and one of the lots of lettuce seed, this retarding effect on germination was rather small. The extract from celery seeds completely inhibited the germination of lettuce seeds and reduced the germination of naked sugar-beet seeds. The extract from Grand Rapids lettuce seed inhibited the germination of lettuce seeds and reduced the germination of naked sugar-beet seeds more than the sugar-beet seed-ball extract with which it was compared. Extract from Grand Rapids lettuce seeds had a very high total nitrogen content, and a relatively large quantity of ammonia was hydrolyzed from it during germination.

TABLE 19. *Some properties of water extracts from seeds of lettuce, celery, spinach, oats, barley, and sugar beets as related to toxicity* [1]

Kind of extract	Osmotic pressure	pH of fresh extract	pH of solution after germination of		Germination of				Total nitrogen [2]	Free NH_3 [3]
					Naked sugar-beet seeds after—		Lettuce seeds after			
			Sugar-beet seeds	Lettuce seeds	48 hours	96 hours	48 hours	96 hours		
	Atmospheres				*Percent*	*Percent*	*Percent*	*Percent*	*Milligrams*	*Milligrams*
Water (check)	0.00	5.57	6.81	4.51	90	98	97	99		
Lettuce (New York No. 12)	1.20	5.50	6.27	5.34	88	98	85	98	0.087	0.006
Lettuce (Grand Rapids)		5.69	8.18	8.39	28	44	0	0	1.628	.336
Celery	4.00	5.97	8.37	8.57	60	68	0	0	.261	.012
Spinach	1.45	6.25	8.12	8.52	82	82	90	93	.122	.011
Oats		5.45	7.70	7.65	88	94	51	87	.254	.008
Barley		5.68	7.65	7.90	88	88	90	92	.103	.006
Sugar beet (U. S. 12 D)	4.28	5.68	8.43	7.65	36	50	0	0	.884	.250

[1] Extracts from dry seeds prepared as previously described for sugar-beet seed-ball extracts.

[2] Measured as milligrams per milliliter of fresh solution.

[3] Measured as nitrogen per milliliter of solution after germination.

SOME PROPERTIES OF PRESSED JUICE FROM FLESHY FRUITS OF TOMATOES, CUCUMBERS, AND MELONS

Tests were run to determine the relationship between the properties of dry-seed extracts and the juice from fleshy fruits. Juice was pressed from the ripened fruits of tomatoes, cantaloups, watermelons, and large green cucumbers. The juices were then filtered and sterilized by heating. Several kinds of seeds, whose tolerance to osmotic pressure had previously been determined, were placed on cotton substrata moistened with the pressed juice. Some of the properties of the prepared juices and their effects on the germination of several kinds of seeds are shown in table 20. It is evident that all of the seeds used in the test were retarded in germination, and furthermore that osmotic pressure appeared to be the major factor responsible. This is in contrast to the relative importance of the two major factors responsible for the toxicity of extracts from sugar-beet seed balls and other dry fruits.

Nitrogen analyses were made of fresh juices of fleshy fruits and of the same juices after they had been recovered from substrata on which seeds had been germinated. Similar determinations were made after incubation with an extract prepared from germinating sugar-beet seeds. The average total ammonium nitrogen on five lots of tomato juice immediately after adding 15 ml. of an extract from germinating sugar-beet seeds to 50 ml. of tomato juice was 0.109 mg. per milliliter. After 3 days' incubation this increased to 0.126 mg. and after 6 days' incubation to 0.130 mg. per milliliter The average pH value on these samples after 6 days' incubation was 4.42. Evidently the buffering capacity (table 20) was sufficiently high to neutralize the free ammonia and render it comparatively ineffective as a toxic agent.

TABLE 20.—*Some properties of pressed juice from the fleshy fruits of tomatoes, cucumbers, and melons, and a comparison of their influence on the germination of seeds with that of salt or sugar solutions of similar osmotic pressure*

Juice or solution used to moisten cotton substratum	Osmotic pressure	Germination of— Naked sugar-beet seeds after 50 hours	Naked sugar-beet seeds after 72 hours	Lettuce seeds after 50 hours	Lettuce seeds after 72 hours	Tomato seeds after 50 hours	Tomato seeds after 72 hours	Cucumber seeds after 50 hours	Cucumber seeds after 72 hours	pH of fresh juice	Total nitrogen[1]	Sodium hydroxide
	Atmospheres	*Percent*	*Per cent*	*Per cent*	*Percent*	*Percent*	*Per cent*	*Per cent*	*Per cent*		*Milligrams*	*Milliliters*
Water (check)		90	96	94	99	18	78	94	94			0.1
Tomato juice	8.76	43	54	0	0	0	0	22		4.20	1.048	29.6
Sugar solution[3]	8.40	67	77	0	0	0	0	28	70			
Cucumber juice	5.77	72	80	0	16	2	2	91	92	5.77	.377	4.4
Salt solution[3]	5.78	69	78	10	11	0	0	92	96			
Cantaloup juice	15.33	16	32	0	0	1	1	0	0	6.01	1.112	7.0
Salt solution[3]	15.55	21	34	0	0	0	0	0	0			
Watermelon juice	12.73	28	32	0	0	0	0	0	0	5.41	.481	3.7
Sugar solution[3]	12.55	40	62	0	0	0	0	0	0			

[1] Measured as milligrams per milliliter of fresh solution

[2] Milliliters of 0.035 normal NaOH required to raise 10 milliliters of pressed juice to pH 9, indicates relative buffering capacity of the pressed juice

[3] Data taken from earlier experiment on the effect of osmotic pressure, therefore direct comparison is not possible, but general trend is very obvious

DISCUSSION

In the rather extensive investigations that numerous workers have made on the factors affecting the germination of seeds in fleshy fruits, the only factor that has received common recognition is osmotic pressure. At various times such indefinite terms as unnamed "specific inhibitors" and "metabolic byproducts of germination" have been postulated as sole or contributory factors adversely affecting germination.

In the present paper the effects of osmotic pressure and other factors affecting seed germination in the presence of water extracts have been evaluated. Although osmotic pressure may be the major factor causing inhibition of germination by fruit juices, evidence is here presented that, in the case of water extracts from the seed envelopes of dry fruits, free ammonia released from nitrogenous compounds in the course of seed germination is a more significant factor.

A summary of the relative importance of the principal factors on which the toxicity of sugar-beet seed-ball extracts was found to be dependent is graphically presented in figure 6. The data, which were taken from table 5, show that free ammonia, pH value, and toxicity are correlated in all cases except one, that of the U. S. 12-C lot grown on extremely saline soil. The very high osmotic pressure in this case was probably a more important factor than the free ammonia. This case also indicates the magnitude of difference in osmotic pressure necessary to depress germination. There was an extreme difference between the osmotic pressure of seed from U. S. 12-D and that of seed from U. S. 12 C. The lack of direct relationship between toxicity and osmotic pressure is indicated by the crossing of the lines in the diagram between the percentage germination and osmotic pressure axes.

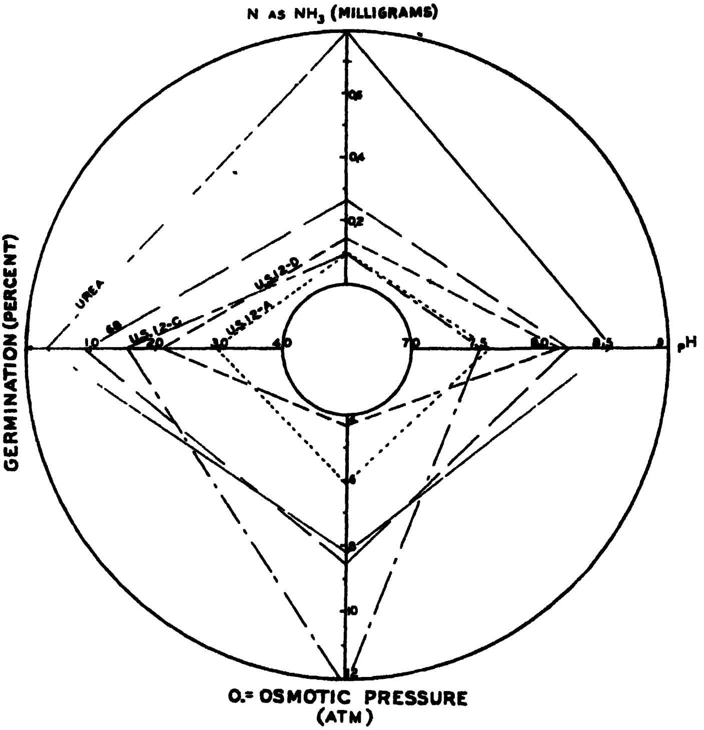

FIGURE 6.- Relation of free ammonia, hydrogen-ion concentration, and osmotic pressure to the toxicity of sugar-beet seed-ball extracts and a solution of urea. Lines drawn from the points on the axes serve to aid interpretation of correlation between factors. The circles indicate boundaries of the figure.

Sufficient ammonia was released by germinating seeds from water extracts of sugar-beet seed balls and certain other dry fruits to account adequately for the toxic effects that have been observed when seeds are germinated in the presence of these extracts. The ammonia was hydrolyzed from the water-soluble nitrogen compounds of seed extracts by both germinating seeds and solutions prepared from germinating seeds. The ammonia released not only neutralized organic acids produced during germination but also caused a sharp rise in the pH of the substratum. In the case of sugar-beet and certain lettuce-seed extracts, 0.3 to 0.4 mg. of nitrogen as ammonia per milliliter of solution was released. Synthetic solutions containing a similar amount of ammonia were shown to be toxic to germinating seeds. Even smaller amounts of ammonia were toxic when the ammonia solution was allowed to flow continuously through the substratum so that the concentration was not reduced by neutralization.

The toxicity of seed extracts and synthetic organic nitrogen solutions was governed not only by the amount of hydrolyzable nitrogen present but also by the rapidity of hydrolysis. Thus asparagin, which was more slowly hydrolyzed than urea, was less toxic than urea during the early stages of germination. Since the hydrolytic agent was present in the immediate vicinity of the seed, the concentration of ammonia was greatest near the seed and the concentration increased as germination progressed. The greater tolerance of radish and cucumber seeds to ammonia solutions was shown to be due more to the production of organic acids than to an actual tolerance to larger amounts of free ammonia. That free ammonia was the toxic agent rather than ammonium salts was shown by the relatively slight inhibition produced by fairly high concentrations of ammonium sulfate. A solution of ammonium sulfate containing 3.5 mg. of nitrogen per milliliter reduced germination less than did a solution of ammonium hydroxide containing 0.3 mg. of nitrogen per milliliter. Still lower concentrations of ammonium hydroxide were effective when the concentration was maintained by a continuous change of solution (fig. 4, *B*).

The present studies showed that ammonia was toxic not only to germinating sugar-beet seeds but also to seedlings and plants that were 2 months old. This is in line with the conclusion of Willis and Rankin (*26*, *27*), who reported that ammonia released from concentrated organic nitrogenous fertilizer applied to the soil was toxic to cotton seedlings. The conclusion of Willis and Rankin was challenged by Tiedjens and Robbins (*24*), who claimed that plants grew as well when ammonium hydroxide was used as the sole source of nitrogen as when some other form such as ammonium sulfate or calcium nitrate was used. However, the pH of the nutrient solutions of Tiedjens and Robbins was evidently very unstable. Starting with an initial pH as high as 9.0 in a continuous-flow experiment, the highest pH value recorded on the solution after passing through the sand culture was 3.8. It is evident that very little free ammonia was maintained in contact with the plants.

Fife and Frampton (*5*) reported that when sugar-beet plants were subjected to high concentrations of carbon dioxide there was a distinct rise in the pH of the juice extracted from the leaf blades owing to the liberation of ammonia from amides and other soluble nitrogenous compounds. They further demonstrated that the amount of ammonia released was dependent on the original nitrogen content of the leaves.

The toxicity of seed extracts varied between species and varieties and between different seed lots of the same variety. This variation was influenced by several factors such as the amount of nitrogen present in the extracts, the rapidity with which it could be hydrolyzed to ammonia, and the buffering capacity of the seed extract. This latter factor was found to be of special importance in the case of fleshy-fruit juices and will be discussed more fully later.

Among the factors that influence nitrogen content of seed are nitrogen nutrition and genetic constitution. Pucher, Curtis, and Vickery (*17*) have shown that betanin, the red pigment of the beet, contains approximately 6 percent of nitrogen. Keller (*8*) has shown that color differences in beets are due to differences in genetic constitution, although environmental factors may also play a part in determining how the genetic factors are expressed. One of the very apparent characteristics of sugar-beet variety 68 was its comparatively high red pigmentation of seed pericarp, which may be the source of some of the nitrogenous compounds that are water soluble and from which ammonia is hydrolyzed during seed germination.

The relation of color to nitrogen content may be more important in the case of lettuce varieties. Of the varieties studied, seed of the Grand Rapids variety was conspicuous by its black color and high nitrogen content. Data reported in this paper indicate that at least twice as much free ammonia was released from the water extract of lettuce seed of the Grand Rapids variety as was necessary greatly to retard or inhibit the germination of lettuce seed in Petri dishes. Knowledge of the fact that relatively large amounts of free ammonia are released from some lettuce-seed extracts offers an explanation for the phenomena reported by Shuck (*20*, *21*). Shuck found that when lots of seed of some varieties of lettuce are successively germinated on the same substratum byproducts of germination accumulate which have an inhibitory action on germination. He stated that when these substances have accumulated to a point at which no more seeds will germinate the substratum can then be washed and seeds will again germinate normally. It is evident that during the germination of successive lots of seed on a moist substratum soluble nitrogen compounds would accumulate so that eventually there would be a condition similar to one where the cotton was moistened with seed extract.

Thompson (*22*) reported that some varieties of lettuce are hard to germinate but that others are germinated readily. He stated that seed of the Grand Rapids variety is generally hard to germinate and gave data to show that this difficulty could be largely overcome by both soaking and drying the seed at low temperature. It is evident that the removal of water-soluble nitrogen during the soaking process would minimize any damage that otherwise might result from the release of ammonia by the germinating seed. Thompson also presented data to show that certain conditions of light and temperature following the soaking or washing treatment further increased germination. The effect of light and temperature may be entirely different from the effect of washing and may have no connection with prevention of damage from free ammonia. Later, Thompson and Kosar (*23*) reported several sulfur compounds such as thiourea, thiosemicarbazide, etc., as stimulating the germination of dormant lettuce seed. The comparable compounds containing oxygen in place of sulfur or hexavalent sulfur such as sulfate were either ineffective or toxic to germination. The nature of the chemicals that stimulated germination, all of which contained divalent sulfur, suggests the possibility that they may have influenced the amount of free ammonia present by either retarding enzymatic hydrolysis or by chemical combination. Tests conducted by the authors have shown that sugar-beet seed balls previously soaked in a solution of thiourea germinate more uniformly and show less injury after long periods of cold storage, such as are used in vernalization tests, than with any other treatment thus far tried. In tests with sugar-beet seed-ball extracts, where the principal inhibiting factors are known to be hydrolyzed free ammonia and osmotic pressure, the addition of thiourea, although it increased the osmotic pressure, reduced the toxic effect, as shown by increased germination percentage and improved appearance of the sprouts of the test seeds.

Much of the inhibitory effect of tomato, cucumber, and melon juice on the germination of sugar-beet, tomato, lettuce, and cucumber seed can be accounted for by the osmotic pressure of the juice. Lavialle (*10*) and Litvinov (*12*) stated that the essential cause of the inhibitory action of all fruit juices is osmotic pressure. When seeds were germinated on a substratum moistened with tomato juice, the low initial pH of the juice and its high buffering capacity prevented the release of free ammonia. Some nitrogenous compounds in the tomato juice were hydrolyzed during germination tests, but the high acidity of the tomato juice held the ammonia in a combined form and prevented it from being a major factor in germination inhibition. Litvinov stated that the active acidity of the tomato juice was not responsible for any inhibiting action, but that on the contrary an increase in inhibition occurred when the pH of the juice was raised to 6.5. It is easy to see that a rise in the initial pH of the tomato juice might make the release of ammonia a more important factor. In fact, tests conducted by the authors showed that tomato juice increased in toxicity as the pH was raised and that sugar-beet seed-ball extracts decreased in toxicity when they were buffered at a low pH.

Ammonium hydroxide solutions seemed to have some effect on germination other than that accounted for by a rise in pH, even though the rise in pH of the substratum on which seed had germinated corresponded rather closely with the extent of injury. It was shown that ammonium sulfate at a low pH was not very toxic, and also that seeds were able to initiate germination and subsequently neutralize sodium hydroxide or sodium carbonate solutions having an initial pH between 11 and 12. By using a continuous flow of fresh solution through the substratum on which seeds were placed and protecting the solutions from any neutralizing influences of gases such as carbon dioxide, it was possible to determine the effect of a maintained pH. The data showed that ammonium hydroxide at pH 9.3 to 9.7 was more toxic to germination and growth than either potassium phosphate or sodium carbonate at a pH of more than 11. Ammonia solutions at a pH slightly above 10 completely inhibited germination. The data also showed that at pH values above 9 the amount of ammonium ion available for release as free ammonia may also be a critical factor in the germination of seeds.

SUMMARY

Studies are reported relating to the properties of dry- and fleshy-fruit extracts that are responsible for toxic and inhibitory effects on germinating seed.

In the case of sugar-beet seed-ball extracts, the toxic effect on germinating seed was shown to be largely due to the toxic action of ammonia, which was released from the nitrogenous compounds of the extracts by enzymatic hydrolysis.

The presence of from 0.3 to 0.4 mg. of free ammonia per milliliter of solution was toxic to germinating seeds, young seedlings, and 2-month-old sugar-beet plants.

Sufficient ammonia was released not only from sugar-beet seed-ball extracts but also from certain other dry-fruit extracts and solutions of urea and asparagin to account for their toxic effect on germinating seeds.

Although the ammonia released from sugar-beet seed balls and some lettuce-seed extracts was sufficient to cause a rise of 4 units in the pH of the solutions, the increase in pH did not completely explain the toxic action of the released ammonia. This was shown by the fact that ammonium hydroxide solutions at pH 9 to 9.5 were more toxic to germinating seeds than either monopotassium phosphate or sodium carbonate solution at pH 11 to 11.5.

The tolerance of various seeds to ammonia solutions and osmotic pressure is reported. There was a marked differential tolerance of the seeds tested to osmotic pressure, but all seeds were sensitive to ammonia solutions containing 0.1 mg. of nitrogen per milliliter when the ammonia concentration was maintained.

In contrast to the major role played by ammonia in the toxic action of dry-fruit extracts, osmotic pressure seemed largely to account for the inhibition to germination of seeds in contact with juices from fleshy fruits. This difference is explained on the basis of the high acid-buffering capacity of fruit juices, which prevented the accumulation of ammonia in the free state. Combined ammonia in the form of salts was shown to be comparatively nontoxic.

The removal of water-soluble nitrogen fractions from the pericarpal tissue affords an explanation of the beneficial effects of washing or soaking some seeds prior to germination tests.

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EFFECT OF CERTAIN ENVIRONMENTAL CONDITIONS ON THE PREVALENCE OF OPHIOBOLUS GRAMINIS IN THE SOIL[1]

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INTRODUCTION

Investigations of fungi and bacteria in soil have shown that the type and number are affected greatly by environment. The effect of environmental factors on the prevalence of soil-borne parasites has received but little attention, in contrast to the extensive studies of the environmental factors affecting the behavior of parasitic organisms in pure culture or in tests of pathogenicity. In the case of soil-borne parasites, information on the behavior in infested soils under various conditions is more important than that on the causal organisms in pure culture.

The experiments reported here were made to determine whether certain environmental conditions, particularly temperature, moisture, and compactness of the soil, favored the retention of *Ophiobolus graminis* Sacc., the parasite that causes take-all of wheat, in naturally infested uncropped soils. For comparison, a few experiments on the tolerance of this organism to extreme temperatures were conducted in pure culture.

EXPERIMENTAL METHODS

In the studies of *Ophiobolus graminis* in pure culture, low temperatures were obtained by both outdoor exposure and artificial refrigeration. Cultures of *O. graminis* were grown on potato-dextrose agar in test tubes. Certain of these cultures were kept in an ordinary thermograph shelter, and others were buried at progressive depths in the soil, where they were exposed to decreasing extremes of temperature. The temperatures were measured with maximum and minimum thermometers and a thermograph. At various intervals during the winter transfers were made to find out whether the fungus was viable.

The thermal death point of *Ophiobolus graminis* growing on potato-dextrose agar was determined by the standard method employed by bacteriologists, with modifications for fungi.

In the tests of infested soils the samples were exposed to various temperatures by outdoor exposure in flats, in the greenhouse in flats and metal pans, and in a freezing chamber in flats; or they were stored in a deep cave in cans. Soil samples described as "moist" were maintained at approximately 60 percent of the maximum water-

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holding capacity, while the dry samples were completely air-dried. Looseness of the soil was maintained by occasional stirring, and compactness by thorough tamping while in a moist condition.

The severity of take-all lesions on Kanred wheat grown in the greenhouse was used as an indication of the presence and extent of infestation in a given sample of soil. The method has been described previously.[2]

EXPERIMENTAL RESULTS

EFFECT OF EXTREME TEMPERATURES ON OPHIOBOLUS GRAMINIS IN PURE CULTURE

Davis[3] showed that *Ophiobolus graminis* grows best at about 23° to 24° C. At 4° growth was exceedingly slight, while at 36° it was checked entirely. The fact that the fungus grows only slightly at 4° C. (39.2° F.) suggested the experiments to determine whether the winter cold in Kansas might not only check its growth but possibly kill it. These experiments were conducted during the winter of 1925–26 and 1926–27.

All the cultures were viable at the end of both winters. The weekly maximum and minimum temperatures in the instrument house, given in table 1, show that the organisms survived temperatures as low as −2° F. Cultures of the fungus also were subjected to repeated alternations of temperature ranging from 70° to as low as −20°. None of the exposures, shown in table 2, affected the viability of the fungus.

Determinations of the thermal death point of *Ophiobolus graminis* showed that both the microhyphae and the macrohyphae were killed when exposed for 10 minutes at 122° F. (50° C.). The fungus remained viable after exposure for 10, 20, and 30 minutes to a temperature of 113° F. (45° C.). Cultures exposed 30 minutes recovered more slowly than those exposed for shorter periods.

TABLE 1.—*Weekly minimum and maximum outdoor temperatures during winter months of 1926 and 1927 when cultures of Ophiobolus graminis were stored outdoors at Manhattan, Kans.*

Year	Month	Experiment No	Temperature							
			First week		Second week		Third week		Fourth week	
			Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
			° F	° F	° F	° F	° F	° F	° F	° F
1926	January	1					54	3	52	0
1926	February	1	56	24	63	20	59	1	68	28
1926	March	1	58	14	70	11	78	20		
1926	December	2			55	−1	48	12	57	16
1927	January	2	64	16	48	−2	36	9	64	14
1927	February	2	62	30	52	5	72	7	70	23
1927	March	2	66	9	68	25	77	23	66	25
1927	April	2	77	30						

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TABLE 2.—*Time and temperature of exposure in a freezing chamber of pure cultures of Ophiobolus graminis*

Period of freezing		Temperature			Period of freezing		Temperature		
Date	Duration	Minimum	Maximum	Mean	Date	Duration	Minimum	Maximum	Mean
1926	*Hours*	*° F.*	*° F.*	*° F.*	*1927*	*Hours*	*° F.*	*° F.*	*° F.*
Dec. 3-4	5.0	[1]−13	10.4	−8.7	Jan. 6-7	16.5	[1]−13.0	30.0	9.5
Dec. 7-8	29.0	[1]−20.2	15.8	−1.1	Jan. 8-15	81.0	[2]−11.2	30.2	9.0
Dec. 9-10	20.5	[1]−16.6	21.2	−1.3	Jan. 18-22	38.0	[2]−7.6	30.2	7.8
Dec. 11-12	2.0	[1]−16.6	−4.0	−11.3	Jan. 31	9.0	[1]−5.8	22.1	4.1
Dec. 13-14	5.5	[1]−13.9	1.4	−5.9	Feb. 10-11	16.5	[2]−5.8	26.6	−.4
Dec. 16-17	25.0	[1]−12.1	21.2	−6.1	Feb. 14-15	11.5	[2]−7.6	−.4	−2.9
Dec. 20-21	9.5	[1]−9.4	10.4	−1.8	Feb. 17	8.5	[2]−2.2	23.0	6.3
					Feb. 19	7.0	[1]−.4	24.8	6.3

[1] Cultures were held at a temperature of approximately 70° F. during the intervals between freezing.
[2] Cultures kept below freezing while the freezing chamber was not operating.

EFFECT OF EXTREME TEMPERATURES AND DROUGHT ON OPHIOBOLUS GRAMINIS IN SOIL

Numerous experiments were conducted in several years to determine whether summer heat and drought would reduce the quantity of *Ophiobolus graminis* in infested soil in the field. These experiments were made by comparing the relative abundance of the fungus in the upper soil layers with that in the deeper layers, which had been subjected to less heat and drought.

The severity tests with Kanred wheat showed that the fungus was reduced somewhat during the summer in the upper 2-inch stratum, but the difference in the abundance of the organism between this stratum and lower depths down to 10 inches was too small to be of practical importance. All the soil strata studied retained sufficient infestation to produce severely diseased wheat plants.

Infested soils were exposed to more severe conditions in the greenhouse. In 1929, 1931, and 1932, soil from infested spots in the field was taken to the greenhouse in the spring, spread in 1-inch layers in shallow pans, and exposed unshaded and without watering until the latter part of October. In 1932 the tests were duplicated with soil samples placed in an elevated position outdoors, where they were protected from the rain but exposed to the sun during the summer. Control samples were stored in a cool cave. The soils were tested for infestation during the winter following the exposure. The results are given in table 3, and temperatures of the soil samples exposed outdoors in 1932 are shown in figure 1. The temperatures of the soil samples stored in the greenhouse are not given, but they were much lower than those of the samples exposed outdoors.

All the soil samples exposed to heat and drought retained enough infestation to produce severely diseased plants, but in three of the four tests the infestation was reduced to some extent. The experiment performed in 1931 showed that soil kept in the greenhouse had more infestation than the control. However, in this experiment the severity rating was high, and when plants are very severely diseased it is difficult to make fine distinctions. Consequently, the apparent difference may not be very significant.

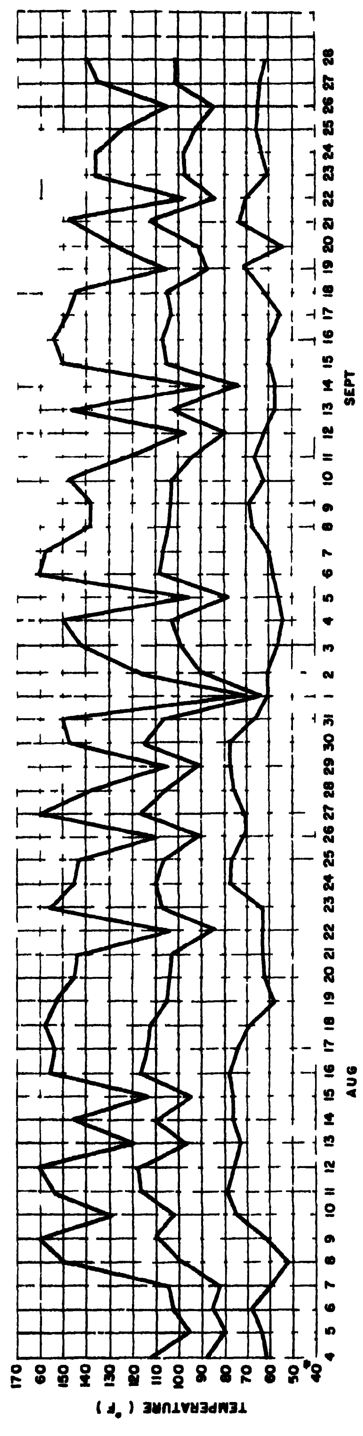

FIGURE 1.—Maximum, minimum, and average daily temperatures of a layer of infested soil 1 inch thick exposed outdoors during August and September 1932

TABLE 3.—*Severity of take-all on wheat plants grown in the greenhouse in infested soil that had been dried in the greenhouse or outdoors, Manhattan, Kans., 1929-32*

Soil treatment and year	Tests	Plants	Average height of plants	Plants tillering	Culms		Plants dead	Plants having diseased—			Roots retained	Average severity of lesions on—			Severity rating
					Number	Heading		Crowns	Culms	Roots		Crowns	Culms	Roots	
	No.	No.	In.	Pct.		Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	
Dried in greenhouse:															
1929	6	105	25.5	48.2	195	9.5	36.1	45.4	25.9	12.1	78.0	67.5	60.0	66.7	20.8
1931	8	70	17.5	27.1	92	8.6	40.0	100.0	96.1	99.7	20.1	90.7	76.8	92.1	148.5
1932	8	59	23.8	13.8	65	35.6	14.8	100.0	32.1	99.6	54.0	69.8	52.4	67.0	48.8
Average[1] and total	22	234	22.2	29.7	352	17.5	30.3	81.8	51.3	80.4	50.7	76.6	63.0	75.5	72.7
Dried outdoors:															
1932	6	55	22.8	50.0	101	41.5	34.5	64.5	48.8	64.1	57.4	77.8	61.8	85.3	38.3
Controls:[2]															
Infested soil:															
1929	6	111	20.2	7.2	124	23.3	28.8	84.9	57.5	79.6	48.3	87.0	74.4	81.1	66.6
1931	9	70	16.2	31.4	118	16.1	44.2	100.0	82.4	98.9	23.5	90.0	67.1	83.2	115.1
1932	4	33	19.5	21.2	10	12.5	6.0	100.0	52.4	99.6	45.0	84.0	36.7	78.0	63.2
Average and total	19	214	18.[illegible]	19.[illegible]	252	17.[illegible]	26.[illegible]	91.9	63.4	92.7	38.9	87.0	59.3	80.8	80.4
Sterilized soil:															
1929	6	98	28.4	40.0	140	59.2	0	0	0	0	100.0	0	0	0	0
1931	10	86	30.6	82.[illegible]	249	53.8	0	0	0	0	100.0	0	0	0	0
1932	6	54	30.8	98.1	218	72.0	0	0	0	0	100.0	0	0	0	0
Average and total	22	238	29.9	70.2	607	61.6	0	0	0	0	100.0	0	0	0	0

[1] Simple averages.

[2] Soil collected from the same spot but not exposed to heat and drought.

Infested soil exposed alternately to growing and freezing temperatures under conditions identical with those of the organism in pure culture showed no reduction of the disease on wheat plants subsequently grown in the soil. Thus, alternate freezing and thawing did not injure the organism in either soil or pure culture. The temperature records are shown in table 2.

In an experiment in which *Ophiobolus graminis* was grown in pure culture it had a thermal death point of 122° F. (50° C.) when exposed for 10 minutes. In another experiment in soil it was not killed at temperatures that ranged from 140° to 160° F. for more than an hour on each of several days. The fact that the macrohyphae on infected plants are much coarser and their walls thicker than in pure culture may explain this difference.

EFFECT OF TEMPERATURE, MOISTURE CONTENT, AND COMPACTNESS OF SOIL ON VIABILITY OF OPHIOBOLUS GRAMINIS

The effect of exposure conditions on *Ophiobolus graminis* either in the absence or in the presence of the host should be reflected later on wheat plants grown in the soil.

The three environmental factors most likely to affect soil flora in the field are moisture, temperature, and compactness or tilth. Accordingly soil from infested spots in the field was exposed for various lengths of time to eight combinations of these three factors. These combinations were: (1) moist, cool, compact; (2) dry, cool, compact; (3) moist, cool, loose; (4) dry, cool, loose; (5) moist, warm, compact; (6) dry, warm, compact; (7) moist, warm, loose; and (8) dry, warm, loose.

The two temperature conditions were obtained by storing one set of soil samples in a deep cave and the other in the greenhouse. The temperature in the cave averaged 37.5° F. and was always above freezing during subzero weather, and in the summer it averaged 71.5°, never going above 72°. The greenhouse temperature was fairly uniform during the winter, with an average of 70.2°, and was variable during the summer but averaged 83.6°. The average maximum daily temperature in June was 97.1°, in July 110.7°, and in August 102.5°. The dry compact samples were so thoroughly puddled and hard at the end of the storage period that a hammer was required to pulverize them. The dry samples were air-dried. Water was applied to the moist samples from time to time. At the end of the storage period all of the soil samples were brought to favorable tilth and moisture, and wheat was grown in them in the greenhouse.

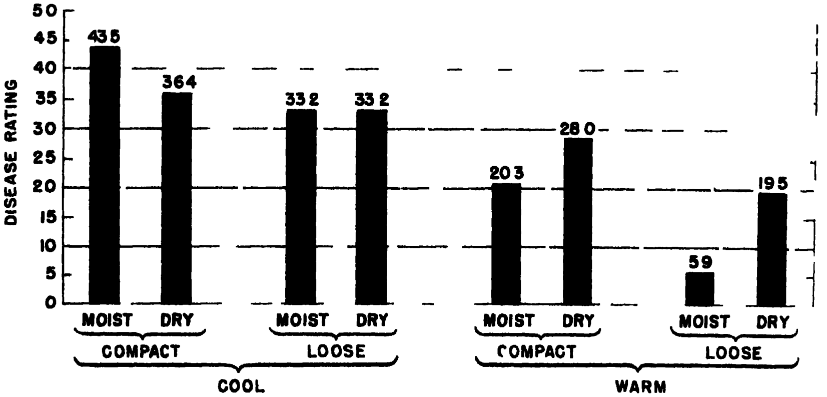

FIGURE 2.—Average severity ratings of take-all on wheat plants grown in the greenhouse in infested soil stored previously under different conditions of moisture, temperature, and compactness, at Manhattan, Kans., 1930–36. (Data in table 4.)

This experiment was conducted four times during the period from 1930 to 1936. For a given experiment all the soil was taken from the same infested spot in the field and was well mixed. Tests for infestation in the first experiment were on plants grown in soil stored for 230 days; in the second experiment, 338 days; in the third, 777 days; and in the fourth, 608 days.

The data on the severity of take-all on the wheat plants grown in the greenhouse in the various soil samples are given in table 4, and the average severity ratings are shown graphically in figure 2. The data in table 4 are summarized in table 5 to show the effects of the environmental factors considered separately and in combinations of two. The relative importance of the factors is indicated by averages and totals from the combinations into which they entered. The severity ratings shown in table 5 are presented graphically in figure 3.

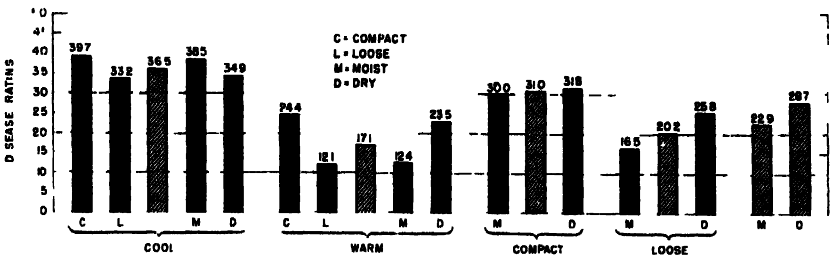

FIGURE 3. – Disease-severity ratings showing the relative effect of single and double environmental factors on the retention of *Ophiobolus graminis* in stored infested soil. The hatched columns represent weighted averages for each single factor. (Data in table 5.)

Under the conditions of the experiments the storage temperature had more effect on the viability of *Ophiobolus graminis* in infested soil than had any other factor. Cool temperature favored the fungus regardless of the other factors. Warm soil combined with other factors in any manner retained less infestation than any similar combination with a cool soil.

TABLE 4.—*Severity of take-all on wheat plants grown in the greenhouse in infested soil previously stored under different conditions of moisture, temperature, and compactness, Manhattan, Kans., 1930–36*

[The length of storage in the various experiments was as follows: No. 1, 230 days; No. 2, 338 days; No. 3, 777 days; and No. 4, 608 days]

Condition of soil in storage	Experiment No.	Lost	Plants	Average height of plants	Plants tillering	Culms			Plants dead	Plants having diseased—			Roots retained	Average severity of lesions on—			Severity rating
						Number	Heading	Average number per tillering plant		Crowns	Culms	Roots		Crowns	Culms	Roots	
Cool, compact		*Number*	*Number*	*Inches*	*Percent*		*Percent*		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Moist	1	4	36	37.2	68.9	99	43.4		0	25.5	15.8	25.4	90.7	58.3	11.6	43.5	10.9
	2	5	46	11.4	43.4	69	0	2.15	36.9	100.0	87.1	99.7	15.2	80.9	44.2	98.3	143.8
	3	5	58	24.0	29.3	81	27.1	2.35	0	100.0	24.5	89.0	49.0	60.4	9.6	70.0	37.9
	4	6	47	16.3	29.7	69	5.7	2.56	12.7	100.0	77.3	98.8	50.8	79.8	45.0	75.0	58.8
Average and total		20	187	22.2	42.8	318	19.0		12.4	81.3	51.1	78.2	51.4	69.8	27.6	71.7	[1]43.5
Dry	1	3	27	34.3	44.4	59	16.9		14.8	100.0	88.8	95.3	35.3	79.6	67.3	84.2	71.2
	2	6	58	17.0	58.6	108	5.0	2.47	37.9	100.0	98.1	99.9	19.7	92.3	70.0	95.3	109.4
	3	5	61	24.4	32.7	88	31.8	2.35	1.6	58.5	4.4	52.4	86.0	50.1	68.3	31.2	15.0
	4	6	52	27.3	21.1	68	66.1	2.45	3.8	16.6	7.4	15.8	91.6	47.7	43.7	75.0	8.1
Average and total		20	198	25.7	39.2	323	29.9		14.5	68.7	49.6	65.8	58.1	67.4	62.3	71.4	[1]36.4
Cool, loose																	
Moist	1	3	28	35.5	50.0	52	17.3		0	16.6	0	6.1	99.1	16.5	0	37.7	4.4
	2	5	56	16.4	28.5	78	2.5	2.37	19.6	100.0	76.0	97.5	18.5	78.5	53.6	89.7	136.5
	3	4	40	23.0	47.5	62	30.6	2.15	0	100.0	0	84.7	74.0	60.1	0	57.7	21.8
	4	6	51	14.3	19.6	61	0	2.00	15.6	100.0	58.8	98.1	62.5	75.4	39.8	58.3	52.1
Average and total		18	175	22.3	36.4	253	12.6		8.8	79.1	33.7	72.1	63.5	57.6	23.3	60.6	33.2
Dry	1	2	21	39.0	66.7	67	26.8		0	20.5	0	2.0	100.0	9.0	0	32.3	3.3
	2	5	46	20.6	52.1	84	4	2.58	10.2	100.0	87.1	99.4	41.2	74.3	41.2	78.8	61.7
	3	4	46	14.2	17.4	55	21.0	2.12	2.1	100.0	18.3	94.5	52.9	76.1	11.4	60.1	42.3
	4	6	50	17.1	8.0	54	25.9	2.00	18.0	43.9	65.3	90.3	56.6	72.0	49.5	75.0	51.8
Average and total		17	163	23.9	36.0	260	20.8		7.5	78.6	42.1	71.5	62.6	57.8	25.5	61.5	[1]33.2

Warm, compact																	
Moist	1	4	37	40.0	72.9	159	26.4		0	0	0	.2	100.0	0	0	35.0	1.1
	2	5	52	10.4	34.6	76	0	2.22	38.4	100.0	55.9	99.9	25.9	83.6	62.8	89.4	124.0
	3	5	52	37.2	44.2	88	64.7	2.60	0	25.0	7.5	20.1	90.1	40.0	18.3	56.1	8.6
	4	6	55	21.0	34.5	82	26.8	2.42	1.8	50.0	16.6	49.1	76.6	75.0	23.8	68.7	18.9
Average and total		20	196	27.1	46.5	405	29.8		10.0	43.7	27.5	42.3	73.1	49.6	26.2	62.3	¹20.3
Dry	1	2	15	27	60.0	31	22.5		20.0	81.2	50.0	52.2	53.8	62.0	86.4	71.5	37.0
	2	6	60	15.1	65.0	136	13.2	2.94	33.3	100.0	91.8	100.0	21.3	74.5	64.8	93.3	85.6
	3	5	60	20.0	36.6	91	57.1	2.40	0	42.0	0	16.1	94.6	36.3	0	30.3	7.8
	4	6	42	26.1	42.8	64	28.1	2.22	0	57.7	26.4	39.6	84.4	34.0	42.5	59.2	13.8
Average and total		19	177	24.4	51.1	322	30.2		13.3	70.2	42.0	51.9	63.5	51.7	48.4	63.5	¹28.0
Warm, loose																	
Moist	1	3	33	39.3	60.6	90	52.2		0	0	0	0	100.0	0	0	0	0
	2	5	39	18.0	100.0	182	24.7	4.66	7.6	74.2	42.5	73.0	50.9	66.2	57.6	62.9	27.5
	3	3	33	33.0	15.1	39	87.1	2.20	0	5.5	0	.6	100.0	5.0	0	2.0	—.8
	4	6	50	23.8	40.0	78	26.8	2.54	0	0	0	Trace	100.0	0	0	0	—25.6
Average and total		17	155	28.5	51.4	389	47.7		1.9	19.9	10.6	18.4	87.7	17.8	14.4	16.2	5.9
Dry	1	3	29	41.6	79.3	104	31.5		0	30.0	3.3	22.1	90.6	7.7	75.0	59.6	8.7
	2	5	48	16.8	56.2	95	17.8	2.74	35.4	100.0	83.3	99.8	27.3	93.6	75.8	99.3	83.2
	3	3	32	32.0	34.3	45	66.6	2.18	0	18.1	0	2.0	100.0	2.0	0	2.0	1.5
	4	5	41	22.2	26.8	59	30.5	2.63	0	54.5	32.4	44.8	80.0	61.4	30.0	59.7	19.1
Average and total		16	150	26.1	49.1	303	36.6		5.8	50.6	29.7	42.1	74.4	48.6	45.2	52.6	¹19.5

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¹ Calculated from averages not an average of severity ratings For method see footnote 2 p 724

TABLE 5.—*Summary of data from table 4 to show the weighted average influence of single environmental factors and of two environmental factors on the prevalence of Ophiobolus graminis in infested soil*

Condition of soil in storage	Tests	Plants	Average height of plants	Plants tillering	Culms		Plants dead	Plants having diseased—			Roots retained	Average severity of lesions on—			Final severity rating
					Number	Heading		Crowns	Culms	Roots		Crowns	Culms	Roots	
Single environmental factor:[1]	*Number*	*Number*	*Inches*	*Percent*		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Cool	75	723	23.5	38.2	1,154	20.5	10.8	76.9	44.1	71.9	58.9	63.1	34.6	66.3	36.5
Warm	72	678	27.0	49.5	1,419	36.0	8.5	46.1	27.4	38.6	74.6	42.0	33.5	48.6	17.1
Compact	79	758	24.8	44.5	1,368	27.3	12.5	65.9	42.5	59.5	61.5	59.7	41.1	67.2	31.0
Loose	68	643	25.7	43.2	1,205	29.4	6.7	57.0	29.0	51.0	72.0	45.4	27.1	47.7	20.2
Moist	75	713	25.0	43.9	1,305	27.2	8.2	56.0	30.7	52.7	68.9	48.7	22.8	52.7	22.9
Dry	72	688	25.5	43.8	1,208	29.3	11.0	67.0	40.8	57.8	64.6	56.5	45.3	62.2	28.7
The two environmental factors:[2]															
Cool:															
Compact	40	385	23.9	40.3	641	24.0	13.4	75.0	50.3	72.0	54.7	68.6	44.9	71.5	39.7
Loose	35	338	23.1	36.2	513	16.7	8.1	78.8	37.9	71.8	63.0	57.7	24.4	61.0	33.2
Moist	38	362	22.2	28.9	571	15.3	10.8	80.2	42.4	75.1	57.4	63.7	25.4	66.1	38.5
Dry	37	361	24.8	37.6	585	25.3	11.0	73.6	45.8	68.6	60.3	62.6	43.9	66.4	34.9
Warm:															
Compact	39	373	25.7	43.8	727	29.8	11.6	56.9	34.7	47.1	68.3	50.6	37.3	67.9	24.4
Loose	33	305	28.3	50.2	692	42.1	5.3	35.2	20.1	30.2	81.0	33.2	29.8	34.4	12.1
Moist	37	351	27.8	48.9	794	38.5	5.9	31.8	19.0	30.3	80.4	33.7	20.3	39.2	12.4
Dry	35	327	26.2	50.1	625	33.4	11.0	60.4	35.8	47.0	68.9	50.1	46.8	58.0	23.5
Compact:															
Moist	40	383	24.6	44.0	723	24.2	11.2	62.5	39.3	60.2	62.2	59.7	26.9	67.0	30.0
Dry	39	375	25.0	45.1	645	30.0	13.9	69.4	45.8	58.8	60.8	59.0	55.3	67.4	31.8
Loose:															
Moist	35	330	25.4	43.9	642	30.1	5.3	49.5	22.1	45.2	75.6	37.7	18.8	38.4	16.5
Dry	33	313	26.0	42.5	563	28.7	8.1	69.6	35.4	56.8	68.5	53.2	35.3	57.0	25.8

[1] Averages and totals derived from all the combinations into which the following single factor entered.
[2] Averages and totals derived from all the combinations into which the following combinations of two factors entered.

Both moisture and compactness modified the effect of temperature on the prevalence of *Ophiobolus graminis* in infested soil. When the infested soil was cool and loose, moisture appeared to be of little importance, because the plants grown in the cool loose soil that had been stored in either a dry or a moist condition had equal disease ratings. A compact warm soil retained more of the fungus in a viable state than a loose one. The addition of moisture to warm soil, either loose or compact, tended to rid the soil of infestation. However, the effect of moisture was greater when the soil was loose.

The individual experiments are all in agreement in indicating that temperature is the most important factor influencing the retention of the parasite in the soil. Some of the results of experiment 1, however, are at variance with those of other experiments with regard to moisture and compactness. This is due perhaps to the fact that the soil in experiment 1 was stored for a much shorter period than in the other experiments.

Infestation did not increase under any condition of storage. In experiments 2, 3, and 4 additional soil was taken to the greenhouse at the time the soil was collected, and wheat was sown at once in order to compare the disease produced initially with that after the soils had been stored. Since the highest retention of infestation was shown to have occurred in the soils stored in a cool condition, this group is used for comparison with the initial infestation in table 6. It is evident that even cool uncropped soil lost some infestation, as might be expected, since Sewell and Melchers[4] have shown that rotation of crops is an aid in controlling the take-all disease.

TABLE 6.—*Comparison of the presence of Ophiobolus graminis in portions of the same infested soil sample, one portion fresh and the other stored*

Experiment No.	Severity rating					
	Stored soil					Fresh soil
	Cool, compact		Cool, loose		Average	
	Moist	Dry	Moist	Dry		
	143.8	109.4	136.5	61.7	112.8	115.1
3	37.9	15.0	21.8	42.3	29.2	63.2
4	58.8	8.1	52.1	51.8	42.7	93.6
Average	80.1	44.1	70.1	51.9		

DISCUSSION

The soil is a common medium for many micro-organisms. The interactions of these organisms may be mutually deleterious, helpful, or neutral. Consequently the results obtained in studies of the effect of soil environment on a single organism are difficult to interpret because of the likelihood of indirect effects of other organisms. Thus the results with *Ophiobolus graminis* just described might have been different if the infested soil had contained a somewhat different microflora.

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[4] SEWELL, M. C., and MELCHERS, L. E. THE EFFECT OF ROTATION AND TILLAGE ON FOOT ROT OF WHEAT IN KANSAS, 1920–1924. Amer. Soc. Agron. Jour. 16: 768–771, illus. 1924.

Aside from the effect of a varying microflora, the effect on the parasite of fluctuations of temperature and moisture within a single year or season should not be great when the infested soil is cropped to a susceptible plant, because an abundance of the host during the growing season provides an opportunity for growth of the fungus to offset the effects of adverse weather conditions. However, there might be a gradual reduction of the organism during a cycle of dry, hot years, or an accelerated accumulation during a cool, moist cycle. It would seem that adverse weather conditions should have the maximum effect on the fungus in infested soil planted to a nonsusceptible crop. Under these conditions hot weather should reduce infestation, the fungus might not be rehabilitated even though cool weather occurred later, and infestation would diminish from year to year.

SUMMARY

Ophiobolus graminis Sacc., the parasite causing take-all of wheat, when in pure culture was not killed by the winter temperatures occurring in Kansas, nor was it affected by repeated abrupt alternations of growing and subfreezing temperatures. The thermal death point of both microhyphae and macrohyphae was about 122° F. (50° C.).

In naturally infested soil the fungus survived temperatures as high as 160° F. (71° C.). High temperatures and drought in the summer reduced the infestation in infested soils only slightly. The abundance of the fungus in infested soils was not reduced by repeated alternations from growing to subfreezing temperatures.

The abundance of the parasite in infested soil was reduced in different degrees by different combinations of moisture, temperature, and compactness of the soil during storage. However, the fungus remained viable in either moist or dry infested soil stored in a warm greenhouse as long as 777 days. In general, cool soil tended to retain the fungus longer than warm soil. The fungus was reduced most in a warm, loose, moist soil, and least in a cool, compact, moist soil.

CHEMICAL COMPOSITION OF SYMPHORICARPOS ROTUNDIFOLIUS AS INFLUENCED BY SOIL, SITE, AND DATE OF COLLECTION[1]

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INTRODUCTION

For a number of years the Utah Agricultural Experiment Station conducted an experiment on the feeding of mineral supplement to range cattle on mountain summer pastures which involved the collection of many of the more important range plant species at regular intervals to determine their chemical composition and thus permit the calculation of the mineral content of the animal diet. Collections of plant material were made at regular calendar intervals, since it was necessary to correlate plant composition with animal reaction as measured at regular time intervals by weight and blood composition. The importance of stage of maturity as a factor in chemical composition was recognized, but, in such heterogeneous range, developmental variations between species and within species made it difficult even to define the growth stage existent at the time of plant selection.

Sampling of vegetation was accomplished by means of a single collection on each collection date. The samples were composited and thus were representative of many areas within the 400-acre pasture. These areas were not regular stations but merely places where the collector happened upon the particular species being collected from the heterogeneous plant cover. Data obtained after several years of such sampling were so variable as to cause some question as to their accuracy. Because of this concern, a sampling experiment was devised to study variability in chemical composition attributable to the following factors: (1) Date of collection, (2) soil type, (3) site or habitat, (4) field sampling technique, and (5) laboratory analysis technique.

LITERATURE REVIEW

Studies correlating natural variation in chemical composition with habitat factors are rare, but there are many papers dealing with seasonal trends and with response to fertilizers.

Of the papers dealing with seasonal trend, a recent report on California foothill range plants is typical. Gordon and Sampson (*6*)[3] showed for herbaceous plants as the season progressed an orderly decline in crude protein, silica-free ash, calcium, phosphorous, and potassium. Crude fiber increased regularly. Deciduous shrubs as the season progressed showed a decline in protein, potassium, and phos-

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[2] Acknowledgment is hereby made of the indispensable assistance given by Dr. A. E. Brandt of the Soil Conservation Service, U. S. Department of Agriculture, in the design and analysis of this experiment, and of the services of the Department of Bacteriology and Biochemistry of the Utah Agricultural Experiment Station in the chemical analyses.

[3] Italic numbers in parentheses refer to Literature Cited, p. 738.

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phorus but an increase in silica-free ash and calcium, and little change in crude fiber.

Stanley and Hodgson (*13*) found regular declines in crude protein and phosphorus as the season progressed and increases in nitrogen-free extracts of Arizona grasses.

Studies in Oklahoma (*4*) showed a close relationship between precipitation and the calcium-phosphorus balance in *Andropogon scoparius*. As precipitation increased calcium content decreased and phosphorus content increased, and as precipitation decreased calcium increased and phosphorus decreased.

Hopper and Nesbitt (*9*) found a quadratic relationship between the April, July, and October composition of grass hay; the July yield being higher in protein and nitrogen-free extract, and lower in fiber. A similar relationship for crude fiber was found in Arizona grasses (*13*).

Orr (*11*), in his classic work on plant minerals, reports positive correlation between mineral content, especially phosphorus, and soil moisture, which, of course, varies with the season. Season influences minerals in that they are highest during periods of maximum leafiness and activity of protoplasm.

Fagan and Milton (*5*) decided that the composition of grasses varied with season largely because of a different leaf-to-stem ratio. McCreary (*10*) suggested that late season increases in total ash and lime might be attributed to dust accumulations.

The response of plant composition to soil was investigated by Truninger and Grünigen (*14*), who studied the relation between soil reaction and phosphorus, potassium, and calcium but found no correlation.

Holtz (*8*) found in oats and certain clovers a relationship between phosphorus and calcium content and the soil upon which they were grown. Peculiarly, the phosphorus content of oats followed the total phosphorus content of the soil, while that of red clover followed more closely the available phosphorus content of the soil. In some soils, calcium content of oats followed the calcium content of the soil but in others no relationship was found.

Orr (*11*) concluded that mineral composition within a species is determined primarily by the soil as shown by the response to fertilizers.

Watkins (*15*) found that *Bromus inermis* when grown in the sun was high in percentage of carbohydrates and low in percentage of nitrogen as compared to plants grown in the shade. Long-day plants as compared to short-day plants were high in percentage of carbohydrates and low in percentage of nitrogen. At bloom stage nitrogen-fertilized plants as compared to check plants were high in percentage of carbohydrate and low in percentage of nitrogen.

Greaves (*7*) found a highly significant positive correlation between total ash and calcium, and total ash and magnesium; between calcium and magnesium; and between phosphorus and crude protein, and phosphorus and crude fat. He found a highly significant negative correlation between total ash and phosphorus, and total ash and calcium and crude fiber and crude fat; between calcium and phosphorus and nitrogen-free extract; and between crude fiber and phosphorus, and crude protein. Similar results have been found in other studies (*3*).

Daniel (*3*), in studies of certain native and cultivated grasses and legumes in Oklahoma, showed positive genetic relationships in the chemical composition of the plants. Thus, the content of plants that are normally low in calcium and phosphorus remained so when the plants were grown on soils rich in these minerals and, conversely, plants normally high in calcium and phosphorus remained so when grown on soil poor in these minerals.

AREA OF STUDY

The investigations described herein were made on high mountain pastures east of Logan, Utah, a region lying within the aspen-Douglas fir zone. The topography is very steep and irregular, the soils highly variable, and the vegetation correspondingly complex. The pastures were not grazed during the season of study.

SOILS

Although no official soil survey has been made of this range-pasture area, the soils were mapped by standard soil survey methods for the purpose of this experiment. Eighteen distinct types were delimited, differing in texture, depth, color, and other profile characters. These types have not been officially described nor given series and type names. Geologically this area is composed of residual sandstones and limestones of the Wasatch conglomerate formation. In many areas the formation has been washed very thin and a substratum of dolomite of the adjacent Nounan formation is very close to the surface.

Three of the more important soil types were selected for use in this study. The basis for selection was the quantity of lime present in the soil as determined by the hydrochloric acid test. Although this was the basis for the selection, numerous other differences were apparent some of which may have been as important as the lime content. It should likewise be noted that total lime content may not be an index to available calcium. Brief descriptions of the three soils, designated A, B, and C, follow.

Soil A.- This type has a surface layer of limefree black soil high in gravel and 3 inches in depth. This layer is underlain to a depth of 72 inches by a red clay high in calcium carbonate. The soil was differentiated from all others by the presence of large quantities of lime in the upper layers, and though occasionally the upper portions of the clay are free from calcium, this is not usual. The subsoils are free from gravel.

Soil B.—This type has a surface layer of black silty clay loam containing some gravel and extending to a depth of 12 to 18 inches. A red clay layer is found under the black surface. Gravel and stones are abundant in local areas but are not common, and abundant calcium is found only in the deep layers beginning at 42- to 54-inch depths.

Soil C.—This soil type is a brownish-black, gravelly, silty clay loam to depths of 12 to 18 inches below which there is a layer of red to yellowish-brown gravelly clay. No calcium carbonate is present, though in local areas limestone was found.

VEGETATION

The vegetation of the pasture belongs chiefly to the sagebrush type or to the aspen type, and both types are of complex composition. In order to eliminate the variable factor of plant-cover type, all sampling was done in the sagebrush type. This type (fig. 1) was analyzed in detail by sample plots and found to have a density (foliage cover) of 20.7 percent. Grasses formed 7.0 percent of the foliage cover; browse plants, 71.1 percent; and forbs, 21.9 percent. A detailed analysis of the composition of the foliage cover follows.

	Percent
Grasses:	
Agropyron pauciflorum	2.0
Bromus tectorum	2.5
Stipa columbiana	.5
Others	2.0
Browse plants:	
Amelanchier alnifolia	3.7
Artemisia tridentata	24.4
Chrysothamnus viscidiflorus	13.9
Prunus melanocarpa	6.7
Purshia tridentata	7.7
Symphoricarpos rotundifolius	14.5
Others	.2

	Percent
Forbs:	
Achillea lanulosa	2.2
Agastache urticifolia	1.3
Aster spp	.8
Balsamorhiza sagittata	.5
Cogswellia macrocarpa	.7
Collinsia tenella	1.2
Crepis acuminata	1.3
Eriogonum heracleoides	3.1
Geranium fremontii	1.2
Lupinus caudatus	2.5
Senecio serra	.5
Stellaria jamesiana	1.4
Vicia americana	.5
Helianthella uniflora	1.7
Wyethia amplexicaulis	1.5
Others	1.2

The grazing season on this area as prescribed by the Forest Service of the United States Department of Agriculture is from about July 1 to October 15.

CLIMATIC CONDITIONS

The climatic conditions of the area studied are typical of those in most of the western mountains Heavy snows remain until about May 1 and the summers are comparatively dry but cool, resulting in good growing conditions throughout a normal summer. The annual precipitation, though not measured, was estimated at 20 inches.

The year during which these studies were made was abnormally dry, adjacent stations showing annual precipitation to be only one-half to two-thirds of normal. There was very little rain during the growing season. Rainfall in May and June was normal, but in July it was only about one-half of normal and all occurred after the samples were collected. Precipitation during August was negligible. September, however, was a near-normal month, with heavy rains about 1 week before the collections were made.

METHODS

Because of the complexity of the vegetation, one species, the roundleaf snowberry (*Symphoricarpos rotundifolius*), a palatable and abundant shrub, was selected for study. This plant was rather uniformly distributed over all soil types and, hence, lent itself well to the study.

It is doubtful whether the reactions of this species would be typical of all other species or, indeed would remain constant for this species from year to year. It was felt, however, that data from this species could be used as an index to the importance that should be accorded a particular factor in collecting plants for analysis.

Collections were made on 3 days in the summer of 1939, July 15, August 15, and September 15. Since normal fruiting did not take place (presumably because of a late frost) it was impossible to describe the stage of maturity except to state that the first collection was considered about full-leaf growth and the last date marked the beginning of leaf dropping.

In addition, from each soil type samples were taken from two sites or habitats, called good and poor. The good sites were simply areas of vigorous or above-average growth, often north exposures or areas where moisture conditions were better. Poor sites were often dry southern exposures and growth was less vigorous. Topography also influenced the difference in growth through concentration of moisture. The difference between the two was not sharp, but, in the field, it was obvious. Variation between these sites could be attributed in part to a difference in developmental stage and, in this respect, might be analogous to seasonal differences within a site.

FIGURE 1.—Sagebrush type upon which collections were made. The aspen type is visible in the background.

The method of collecting material for analysis was designed particularly to eliminate personal error by the following procedure. Transects were considered to be 100 paces along predecided lines. On each soil and site at each date numbers were drawn to determine at which paces along the transect plant material would be collected. Duplicate collections were made within each 100-pace transect. At two points thus selected, and these were marked "a" and "b" in the order of collection. All "a" collections and all "b" collections from 10 such transects were composited, each constituting exactly one-tenth of the total sample. In collecting, the closest plant to the selected point was harvested, all current growth being included.

Thus at each of three collection dates (early, medium, late) duplicate samples were collected from 3 soil types (A, B, and C), and from each soil type on 2 sites (good, poor), giving a total of 12 separate samples at each date or 36 for the season. Each of these samples was, of course, a composite of collections from 10 subareas. Chemical analyses were made in duplicate without regard to their checking, and each analysis was recorded separately to make possible the calculation of an analysis error. Standard analyses (*1*) were made for total ash, calcium, magnesium, phosphorus, crude protein, crude fat, crude fiber, and nitrogen-free extract. On the whole the experiment was set up on the basis of a factorial design. Although there were only 2 direct replications, there was considerable hidden replication, and as a result each conclusion is founded upon at least 12 replications.

Data were studied by the analysis of variance. Individual sums of squares were computed for each degree of freedom (*12, p. 336*). This method of computing is somewhat empirical and involves selected comparisons and, hence, cannot be expressed by a standard equation. In result, it does not differ from the common methods of determining variance except that it permits a subdivision of the variance according to source.

As an example, let us suppose X_1, X_2, and X_3 to be the three observed variables and it is desired to compare the first with the second and the first two with the third. The two comparisons would be represented thus:

$$\frac{(X_1-X_2)^2}{1^2+1^2} \tag{1}$$

$$\frac{(X_1+X_2-2X_3)^2}{1^2+1^2+2^2} \tag{2}$$

The resultant sums of squares are divided by the degrees of freedom to obtain mean squares for each comparison (see table 4).

Through the use of this method it was possible to measure tendencies toward either linear or quadratic relationships between composition and date of collection and between composition and soils. Since but three points were determined for each curve, the data merely measure general trends. For general tests of significance the *F* table of Snedecor (*12*) was used, and unless otherwise specified, interactions were grouped to form the error term.

ANALYTICAL DATA

PROTEIN

A summary of the results of the protein analyses is presented in table 1. Protein showed a highly significant [4] decline as the season progressed, the variation with time being strongly linear.[5] The decline was from an average of 11.24 percent on July 15 to 9.19 on August 15 and to 6.58 percent on September 15. Date of collection was far more important than any other factor in its influence upon protein. Site generally had a significant, though slight, effect upon protein, the good sites yielding an average of 9.31 percent and the poor an average of 8.69 percent. This relationship ceased to exist in late season, however, for there was no significant difference between the two sites in September.

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[4] An effect or difference is said to be significant if it is of such magnitude that the probability of its chance occurrence in undifferentiated material is equal to or less than 0.05. If the probability is equal to or less than 0.01 the effect is highly significant.

[5] Mean square values are shown in table 4.

TABLE 1 — *Chemical composition (air-dry-weight basis) of snowberry plants collected on three different dates, as affected by soil type and site, data based on an average of four analyses*

Collection date	Composition of plants grown on— A soil, Good site	A soil, Poor site	B soil, Good site	B soil, Poor site	C soil, Good site	C soil, Poor site	Average
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
PROTEIN CONTENT							
July 15	11 99	10 67	12 41	10 83	11 11	10 45	11 24
Aug 15	9 75	8 47	9 78	9 5	9 00	8 57	9 19
Sept 15	6 71	6 49	6 4	6 47	6 63	6 77	6 58
Average	9 48	8 54	9 55	8 95	8 91	8 60	
Average for both sites	9 01		9 25		8 75		9 00
NITROGEN-FREE EXTRACT CONTENT							
July 15	47 13	48 56	48 87	50 04	46 89	49 47	48 41
Aug 15	47 88	48 89	45 84	47 77	47 88	47 45	47 62
Sept 15	43 40	43 83	42 02	43 81	44 17	44 13	43 56
Average	46 11	47 09	45 58	47 21	46 31	47 02	
Average for both sites	46 61		46 39		46 66		46 56
CRUDE FIBER CONTENT							
July 15	16 20	15 37	13 85	13 83	16 76	14 09	14 98
Aug 15	16 43	18 29	18 41	17 02	17 80	16 38	17 39
Sept 15	25 13	26 11	27 31	2 76	25 34	22 77	25 42
Average	19 25	19 93	19 88	18 87	19 90	17 75	
Average for both sites	19 59		19 37		18 82		19 26
CRUDE FAT CONTENT							
July 15	11 16	11 80	11 18	12 13	11 2	12 07	11 58
Aug 15	11 90	11 20	12 21	12 27	11 70	12 64	12 00
Sept 15	11 7	11 14	12 32	11 [illegible]	11 34	13 44	11 93
Average	11 63	11 38	11 90	11 99	11 42	12 72	
Average for both sites	11 50		11 9		12 07		11 84
TOTAL ASH CONTENT							
July 15	6 65	6 59	6 73	6 48	7 41	7 06	6 83
Aug 15	6 55	6 42	6 65	6 26	6 75	7 61	6 7
Sept 15	5 41	5 15	5 10	5 18	5 83	5 27	5 32
Average	6 30	6 05	6 18	5 9	6 66	6 65	
Average for both sites	6 18		6 07		6 66		6 30
PHOSPHORUS CONTENT							
July 15	0 231	0 259	0 301	0 266	0 372	0 252	0 286
Aug 15	150	201	208	178	297	429	241
Sept 15	147	163	156	151	295	181	182
Average	177	218	222	198	321	287	
Average for both sites	0 197		0 210		0 304		237

TABLE 1.—*Chemical composition (air-dry-weight basis) of snowberry plants collected on three different dates, as affected by soil type and site, data based on an average of four analyses*—Continued

CALCIUM CONTENT

Collection date	Composition of plants grown on						Average
	A soil		B soil		C soil		
	Good site	Poor site	Good site	Poor site	Good site	Poor site	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
July 15	1.43	1.52	1.36	1.37	1.31	1.48	1.41
Aug 15	1.51	1.72	1.61	1.52	1.62	1.63	1.60
Sept 15	1.39	1.51	1.36	1.32	1.32	1.50	1.41
Average	1.44	1.58	1.44	1.40	1.42	1.57	
Average for both sites	1.51		1.42		1.49		1.47

MAGNESIUM CONTENT

Collection date	A soil Good site	A soil Poor site	B soil Good site	B soil Poor site	C soil Good site	C soil Poor site	Average
July 15	0.319	0.424	0.331	0.390	0.318	0.368	0.358
Aug 15	.262	.302	.303	.309	.288	.293	.293
Sept 15	.295	.328	.250	.286	.245	.316	.287
Average	.292	.351	.295	.328	.281	.326	
Average for both sites	0.321		0.311		0.305		.313

There was an insignificant quadratic relationship between soil and protein, the B soil being more favorable for protein than either the A or the C soil. This relationship was especially obvious in the August analysis but entirely absent in the September analysis. However, the interaction was not significant.

NITROGEN-FREE EXTRACT

Date of collection was far more important than any other factor in its influence upon nitrogen-free extracts. The pronounced decline as season progressed was significantly greater during the last month than during the first month (table 1); in fact, in the A soil there was a slight increase between July and August.

Nitrogen-free extract bore no significant relation either linear or quadratic to soil type; the soil types responded differently at different dates, the B soil appearing to have a greater seasonal response than either the A or the C soil. Site had a significant, though not large, influence, the average percent being 46.01 on good sites and 47.10 on poor sites.

CRUDE FIBER

A very highly significant increase was found in crude fiber as the season progressed and, though the relationship appears to be linear, there was a greater increase during the last month than during the first (table 1).

An insignificant positive relationship existed between lime content of soil and crude fiber yield from plants collected on poor sites, the relationship being essentially linear.

Analysis brings to light an interesting interaction between date of collection and soil in their influence upon crude fiber. The B soil was much more reactive to date than either the A or the C soil. Plants from the B soil contained less fiber at the first analysis and more at the last than those from either of the other soils. A slightly greater fiber content was found in plants from good sites (19.68 percent) than in plants from poor sites (18.56 percent).

CRUDE FAT

Crude fat did not differ significantly with changes in soil, date, or site. However, the data indicated a close relationship between fat content of forage and soil type (table 1). This was especially evident on poor sites.

A small but not quite significant relationship existed between site and fat content of the forage. Poor sites averaged 12.03 percent fat and good sites 11.65 percent.

TOTAL ASH

The total ash content was found to decrease as the season progressed, the decrease being significantly greater in the last month than in the first (table 1).

There was likewise a highly significant relation between soil and total ash content, the C soil averaging more than either the A or the B soil.

Site was insignificantly related to ash content, the poor site averaging 6.22 percent and the good site 6.38 percent.

PHOSPHORUS

Phosphorus showed a highly significant relation to collection date and soil but not to site. Average yields shown in table 1 indicate a tendency toward a large linear decrease as the season progressed.

The phosphorus content was significantly greater on the C soil, especially on good sites. The difference was significantly greater between C and B soils than between B and A soils. Phosphorus was higher on good sites at early and late dates of collection, but higher on poor sites at the intermediate date. Poor sites were higher in phosphorus on A soil but were lower in phosphorus on C soil. This soil-site interaction was highly significant.

CALCIUM

There was no significant difference in calcium content of the plant on different soil types (table 1). A highly significant increase was found in calcium at the middle date and this was followed by a decline, no significant difference existing between the early season and the late season analysis.

Plants from good sites tended to have slightly less calcium (1.43 percent) than those from poor sites (1.52 percent). However, the difference was not quite significant.

MAGNESIUM

Magnesium showed no significant relation to soil (table 1). There was a highly significant tendency toward decrease in average magnesium content as the season progressed. This was exhibited much more strongly in plants from good sites than in those from poor.

Plants from poor sites contained a highly significantly greater quantity of magnesium, averaging 0.335 percent as compared to 0.290 in plants from good sites.

COMPARISON OF FIELD ERROR AND ANALYSIS ERROR

In order to place field errors on a comparable basis the variance of field error of each constituent was divided by the mean of the percent of that constituent in all samples. The same treatment was followed for analysis error. The results are shown in table 2.

From these values it is evident that a generally higher variability occurs between duplicate collection samples than between duplicate chemical analyses. The chemical analysis is most variable in crude fat, nitrogen-free extract, and crude fiber in the order named. This might be considered as an index to the accuracy of analytical methods used. The field-collection duplicates varied greatly in crude fiber, nitrogen-free extract, and crude fat in the order named.

Only in the case of crude fat did the variation within chemical-analysis duplicates exceed the variation between collection duplicates. This would indicate a refinement of analytical method insufficient to differentiate between collection duplicates. This same situation is approached in the case of nitrogen-free extract.

The greater variation shown in some constituents than in others between duplicate collections would indicate that these constituents vary more in individual plants, though it must be kept in mind that chemical-analysis error is carried over to field-collection error. For example, the high field error in the case of crude fat might be accounted for entirely by chemical error.

TABLE 2.—*Field- and laboratory-error comparison (variance divided by general mean) and ratio of laboratory error to field error by constituents of snowberry plants, based on data in table 1*

Constituent	Field error	Laboratory error	Ratio of laboratory error to field error	Constituent	Field error	Laboratory error	Ratio of laboratory error to field error
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
Protein	1.77	0.09	5.84	Total ash	4.57	.02	.44
Nitrogen-free extract	8.06	2.62	32.50	Phosphorus	.42	.004	.95
Crude fiber	18.08	.72	3.98	Calcium	1.89	.00	.00
Crude fat	6.90	10.93	158.40	Magnesium	2.75	.06	2.18

NEED OF DUPLICATE SAMPLING

Estimated standard errors were determined according to the method suggested by Bartlett and Greenhill (*2*). Table 3 shows these estimated errors for each constituent had field and laboratory duplication of various combinations been used. Mean square values derived from table 1 are shown in table 4.

With the exception of crude fat, there is little increase in accuracy gained by duplicate chemical analyses. As Bartlett and Greenhill have stated, "the chief justification of duplicate determinations * * * is that they serve as a check against the possible return of an erroneous figure." In the case of crude fat, however, there is apparent a large variation in chemical analyses, and duplication materially reduces the standard error.

Field-collection duplication generally does not give a significantly increased accuracy of data Duplication of the field sampling, however, does appear to be more important than duplication of laboratory analyses In the case of magnesium, duplicate collection results in a materially decreased standard error but in no other instance does the increased accuracy appear sufficient to justify the labor and cost involved These conclusions do not, of course, ignore the importance of duplication as a means of checking errors

TABLE 3 *Estimated standard errors for each constituent of snowberry plants expressed as percentage of the general mean for various combinations of field and laboratory duplication based on data in table 1*

Field samples	Laboratory samples	Protein	Nitrogen free extract	Crude fiber	Crude fat [1]	Total ash	Phosphorus	Calcium	Magnesium
Number	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	1	22 84	7 84	24 89	9 00	13 24	32 37	10 96	21 04
1	2	22 83	7 60	24 8	7 90	13 24	32 37	10 96	21 04
2	1	22 74	7 59	24 43	9 08	12 54	31 68	9 37	14 60
2	2	22 73	7 33	24 40	6 58	12 74	31 68	9 37	14 60

[1] The larger standard error obtained for crude fat with 2 field samples probably is not significant and it means simply that the values obtained from 2 samples are no more reliable than those obtained from 1 sample

TABLE 4 *Mean square values for each constituent of snowberry plants by comparisons, based on data in table 1*

Comparison	Degrees of freedom	Protein mean square	Nitrogen free extract mean square	Crude fiber mean square	Crude fat, mean square	Ash mean square	Phosphorus mean square	Calcium, mean square	Magnesium mean square
Month linear	1	200 634	292 319	1 305 830	1 394	27 271	0 129	0 000	0 062
Month quadratic	1	846	10 768	12 142	887	7 312	010	779	014
Soil linear	1	364	011	7 192	3 752	2 750	063	052	003
Soil quadratic	1	2 641	1 00	506	405	1 861	090	103	000
Site	1	8 168	21 727	12 194	2 634	467	015	124	037
Month×soil	4	880	6 713	9 103	383	091	002	043	003
Month×site	2	2 127	1 65	1 192	894	429	001	008	004
Soil×site	2	950	1 387	207	751	091	031	067	001
Month×soil×site	4	488	2 20	2 135	1 346	337	001	030	001
Σ Interaction	12	1 003	3 498	4 094	850	230	004	037	002
Field error	18	159	3 757	3 483	818	288	001	028	009
Laboratory error	36	008	1 218	139	1 294	001	000	000	000

CONCLUSIONS

From these studies it is concluded that in collecting plants for complete chemical analysis attention must be given to soil type, site, and date of collection. The importance of these variables, however, is dependent upon the individual constituent being analyzed In collections to determine seasonal variation it is essential to sample within the same site and soil type or so to composite the collection as to make it representative of these two variables In complete chemical analysis the complicating interactions that occur between these variables make it impossible to forecast chemical response to any one factor unless all others are made constant or made a part of the analysis through appropriate experimental design

For these reasons it is suggested that in sampling highly variable range floras for chemical analysis, seasonal collections be made from regularly established stations. These stations should be so located as to be representative of the soil types and sites that exist within the range. This can be done by making the number of stations within each soil type and site numerically proportionate to the area occupied by that particular soil type and site in the entire range. The sample should, of course, be equally composited from each station.

SUMMARY

Chemical response was studied in *Symphoricarpos rotundifolius* near Logan, Utah, to variation in site, date of collection, and soil type.

Date of collection was by far the most influential factor, late season resulting in a highly significant decrease in protein, nitrogen-free extract, total ash, phosphorus, and magnesium; and a highly significant quadratic influence upon calcium. Only crude fat failed to respond to season.

Soil type had a highly significant influence upon total ash and phosphorus content and a pronounced though insignificant effect upon protein content.

Site had a significant influence upon protein and a highly significant influence upon magnesium, protein being higher and magnesium lower on good sites. Nitrogen-free extract was also significantly higher on poor sites.

Complex interactions are common between date of collection, site, and soil, and for this reason attention must be given to all before broad conclusions are drawn regarding the influence of any.

Large error or variation was apparent in the analytical procedure for crude fat, and the field variability was especially high in crude fiber, nitrogen-free extract, and crude fat.

For the determination of most components duplicate collection and duplicate analysis do not increase the accuracy commensurate with the extra expense incurred, and hence can be justified only as a means of checking errors.

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AN INVERSION, A RECIPROCAL TRANSLOCATION, TRISOMICS, AND TETRAPLOIDS IN BARLEY[1]

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INTRODUCTION

The objectives of the investigation reported herein were (1) to explore the possibilities of barley (*Hordeum vulgare* L.) as a subject and source of problems for cytological study and (2) to make a survey of some mutants, varieties, and hybrids of barley in order to determine whether there may be any cytological basis for observed peculiarities, such as dwarfness or sterility, of certain plants. Except in a few instances, the observations here reported were made during June 1940 at Aberdeen, Idaho.

REVIEW OF LITERATURE

Though barley has been used rather extensively in genetic studies, few cytological observations have been reported. The first observers were interested in the number of chromosomes and their morphology in the various species. A review of the contributions of most of the early authors may be found in Ghimpu (*4*)[3] and Lewitsky (*10*).

Cultivated barleys, and some noncultivated species, have 14 somatic chromosomes. Wild species with 14, 28, and 42 chromosomes were reported by Griffee (*5*), Ghimpu (*4*), and others. The chromosomes are long and slender, with median or submedian centromeres. Emme (*3*) was the first to report satellites in barley. Lewitsky (*10*) reported that there were regularly 2 pairs of chromosomes with satellites in the species having 14 chromosomes. One pair of satellites was larger than the other. These satellites are presumably different from the large appendage observed by Hasegawa (*6*) in 9 species from *Aegilops*, *Triticum*, *Secale*, and *Hordeum*. If that is the case, there are 3 pairs of chromosomes with distinguishable appendages.

Johansen (*7*) examined root tips from a commercial lot of seed of *Hordeum vulgare* and found a high frequency (possibly 10 percent) of haploids. He observed two appendages (with inconclusive evidence of two more) in diploid and two in haploid root tips. The haploids were discarded before their constitution was known, so a study of meiosis could not be made in them. Tometorp (*23*) studied meiosis in pollen mother cells of a haploid of *H. distichum*, however, and found occasional bivalents. Usually the chromosomes were dis-

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[3] Italic numbers in parentheses refer to Literature Cited, p. 749.

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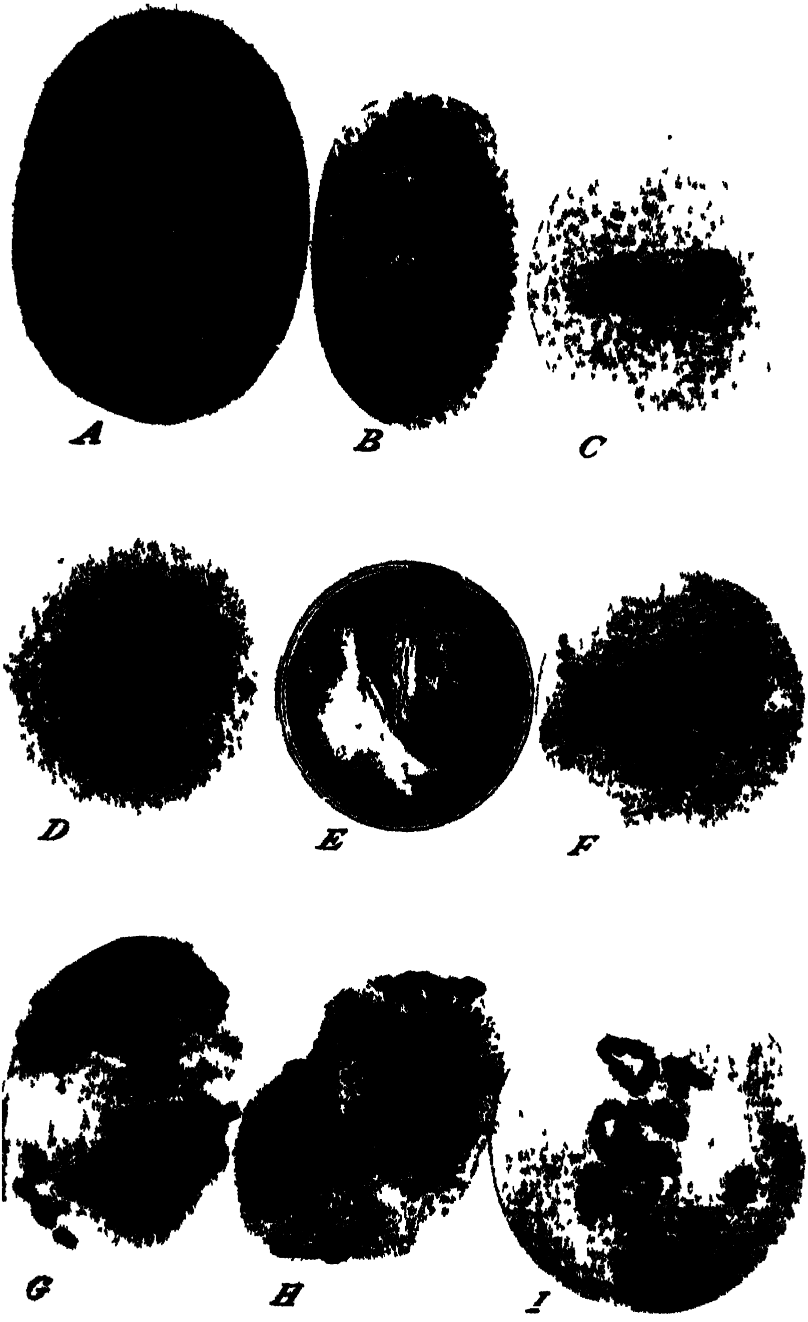

(See Explanatory Legend on opposite page) tributed at random in the first division and the chromatids did not ordinarily separate until the second division. Microspores were observed with 7, 8, and 13 or 14 chromosomes at the first mitotic division.

Ekstrand (*2*) reported observations on the meiotic process in a partially asynaptic strain of barley, and Swenson (*22*) described meiosis in normal plants.

Peto (*13*) examined diploid and tetraploid florets on the same spike for chromosome pairing and chiasma frequency. He found an average of 3.58 quadrivalents (range 1 to 7) in 4*n* pollen mother cells. One of two megaspore mother cells had 7 quadrivalents; the other had 4. In the tetraploid pollen mother cells there were 1.87 times as many chiasmata as in the diploid.

Müntzing, Tometorp, and Mundt-Petersen (*12*) and Karpechenko (*8*), with heat, and Dorsey (*1*), with colchicine, obtained tetraploid barley plants. All found plants with 1, 2, or 3 more than 28 somatic chromosomes in the progeny of tetraploid parents. Müntzing reported 4, and Karpechenko 1, offspring with only 27 chromosomes.

Kattermann (*9*) was the first to report a trisomic plant in barley. He observed mitotic and meiotic divisions in a trisomic plant of *Hordeum distichum*. The chromosome involved was not one with a satellite.

MATERIAL AND METHODS

The plants studied were mainly from varieties and strains of *Hordeum vulgare* that were being used in the barley breeding project of the Division of Cereal Crops and Diseases. An adapted acetocarmine smear technique was used in making all slides for cytological observations. Carnoy's fluid was used as a fixative. Heads were fixed for 1 to several days; root tips for a week or longer. A more complete description of the root-tip smear procedure has been published by Smith (*18*). Some smears were made permanent by an improved method (employing tertiary butyl alcohol instead of xylene) suggested by Sears (*17*). Photomicrographs were taken with a Leitz Makam camera.

A, A bridge with a fragment beside it; first anaphase in a pollen mother cell. *B*, A bridge and fragment at second telophase in one cell of a dyad. The fragment is indicated near the pole. *C*, A ring of four chromosomes (open) at first metaphase in a pollen mother cell of a plant heterozygous for a reciprocal translocation. *D*, Seven pairs at first metaphase from the same slide as *C*. Two pairs are attached at only one end. *E*, First division in a microspore, showing satellites on two chromosomes. *F*, First meiotic metaphase in a pollen mother cell of trisomic plant 202 1; trisome indicated by arrow. *G*, Second meiotic anaphase in a pollen mother cell of trisomic plant 202 2. A lagging chromatid is in each daughter cell. The chromatids of the extra chromosome presumably separated in the first division. *H*, A chromatid in which "misdivision" has occurred; second meiotic anaphase in trisomic plant 202 2. *I*, Diakinesis in a tetraploid "Big Boy," barley. There are three open rings of four, a zigzag group of four (top, right), a chain of four (center, right), and four bivalents. Note the two nucleoli. (Photomicrographs *A* and *B* were taken at a magnification of 1,050 diameters, the others at 850. All were reduced about 1/10 in reproduction.)

AN INVERSION

In attempting to determine the cause of the low seed production in a "Sterile Flowers" stock (a selection from *Hordeum deficiens nudideficiens*, C. I.[4] No. 2229), two of five plants examined in one progeny were found to have seven pairs of chromosomes at first metaphase and bridges and fragments at first and second anaphase or telophase in pollen mother cells (pl. 1, *A* and *B*). Other "Sterile Flowers" plants did not have the bridges and fragments, so presumably there is some other cause for the sterility in "Sterile Flowers" plants. Table 1 summarizes the observations on bridges and fragments at the first division in the two plants. In plant 219–2, 24 percent of the cells had either a bridge or both a bridge and a fragment, and in plant 219–4 the value was 14 percent. A larger proportion of the cells of plant 219–4 were without visible fragments.

At second anaphase and early telophase in plant 219–2, 4 quartets had a bridge and fragment; 16 had a bridge but no visible fragment; and 72 had neither bridge nor fragment. Thus about 20 percent of the quartets had a bridge. The fragment was not necessarily located near the bridge. The fragment presumably was formed in the prophase of the first division, though it may not have become free until the second.

TABLE 1.—*Data on bridges and fragments at the first meiotic division in pollen mother cells of plants with an inversion*

Plant	Cells with a bridge		Cells without a bridge		Total cells
	With a fragment	Without a fragment	With a fragment	Without a fragment	
	Number	*Number*	*Number*	*Number*	*Number*
219-2	18	5	5	67	95
219-4	8	21	9	170	208

The bridges and fragments in the two plants may be accounted for by assuming single cross-overs in an inverted segment, as depicted in figure 1, though, as shown by Richardson (*15*), a duplication can also give bridges and fragments in plants with bivalents only. As the fragment is small the inversion must be near the end of the chromosome. The percentage of bridges, however, might lead one to expect a larger fragment. The explanation may be a high frequency of crossing over localized in the short inverted segment. The bridges at second anaphase and telophase fit the interpretation of single cross-overs in the inverted region, accompanied by simultaneous cross-overs in the proximal uninverted portion of the arm. Explanations for the absence of a fragment in a cell with a bridge at the first division have been considered by McClintock (*11*), Swanson (*21*), and others.

Plant 219–2 (a 2-rowed type) had a seed in all but 1 of 45 primary florets, so female fertility was not much reduced.

McClintock[5] has also observed that inversions (and other aberrations which give rise to bridge configurations at meiosis) do not produce female sterility proportionate to the number of pollen mother cells with bridges.

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[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases.

[5] McCLINTOCK, B. Unpublished observations.

Sturtevant and Beadle (*19*) and others have observed that single cross-overs in heterozygous inversions in *Drosophila melanogaster* do not result in inviable zygotes. Sturtevant and Beadle suggested that the chromosomes were oriented in such a manner that only non-cross-over chromatids get into the nuclei that become the egg nuclei. The same sort of behavior may account for the lack of sterility in the barley plant.

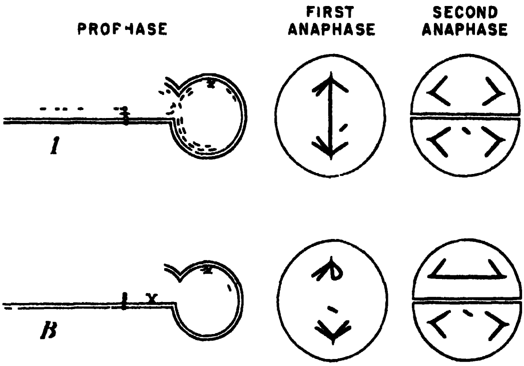

FIGURE 1 — A diagram illustrating some modes of origin of bridges and fragments from crossing over in a bivalent heterozygous for an inversion *A*, A single cross-over in an inverted segment *B*, A single cross-over in an inverted segment and a simultaneous cross-over in a proximal uninverted segment producing a three-strand double cross-over Cross-overs are represented by "x" and the centromeres by circles For more complete diagrams of crossing over in inversions, see McClintock (*11*), Richardson (*15*), and Sax (*16*)

A RECIPROCAL TRANSLOCATION

In 1938 the writer received from G. A. Wiebe[6] seed of plants, some of which were supposed to be heterozygous for a gene causing "Contabescent Anther," a mutant characterized by nearly complete male and female sterility. This mutant was selected from C. I. No. 3845, a variety obtained from Kashmir, India. The sterility is associated with a meiotic irregularity as yet undescribed. In an examination of this stock for the meiotic disturbance, the reciprocal translocation described in this section and the three trisomic plants reported in the next section were found. No cause for the coincidence of these three different irregularities in the same stock was observed. Several normal plants were regular at meiosis.

The translocation apparently involves small segments of each of two nonhomologous chromosomes. Often the configuration at first metaphase is a chain of four instead of a ring, and not infrequently seven pairs are formed (pl. 1, *C* and *D*, and table 2).

More pollen mother cells had zigzag (disjunctional) than open (nondisjunctional) configurations of the chromosomes in the ring or chain (table 2). Plant 201-1 was the parent of 207-1, and the behavior of the ring in the two plants was similar. The behavior of

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[6] Agronomist, Division of Cereal Crops and Diseases.

the ring in the two sib plants of 203, whose grandparent was the parent of 201, differed from that of the ring in 201 and 207 in that fewer rings and chains were formed and more cells had seven pairs. It is possible that another interchange is involved, though the close relationship between 201, 207, and 203 makes this unlikely. Also interchanges involving such small segments that chains and even pairs are formed have not been common in other organisms. Except for the closed rings in the two plants of 203, these plants might be suspected of having received deficiency-duplication gametes formed by nondisjunctional segregations of the ring observed in 201 and 207.

TABLE 2. *Configurations of chromosomes at first meiotic metaphase in pollen mother cells of plants heterozygous for a reciprocal translocation*[1]

Plant	Ring of 4			Chain of 4			7 pairs	Unclassified	Total cells
	Zigzag	Open	Unclassified	Zigzag	Open	Unclassified			
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
201-1	83	48	2	39	19	2	12	16	221
207-1	52	29	1	99	37	7	54	39	318
203-1	2	2	0	34	23	0	119	48	228
203-2	8	12	0	32	21	0	31	122	226

[1] See text for relationship of plants

No comparison of the seed set of normal and ring-forming sib plants has been made as yet, but it is clear from table 3 that plants with the interchange are not "semisterile" in seed set. The first two plants in table 3 were ring-forming plants grown in the greenhouse at Columbia, Mo. The third was grown in the field at Aberdeen, Idaho. The last two plants were normal field plants which were not sibs of the ring-forming plant but do give some information on fertility of plants of similar type. There is a close agreement in the fertility of the three ring-forming plants (65.6, 69.0, and 64.8 percent) and a similar agreement between the two seven-pair plants (92.4 and 93.0 percent). The fertility of the ring-forming plants also approximates the proportion of the pollen mother cells of the first two plants of table 2, in which the chromosomes were oriented disjunctionally.

TABLE 3. *Fertility of ring-forming and normal plants*

Plant	Configuration	Florets	Seed	Fertility
		Number	*Number*	*Percent*
347-1	5 pairs + R_4	96	63	65.6
347-2	do	129	89	69.0
207-2	do	270	175	64.8
216-2	7 pairs	198	183	92.4
217-1	do	213	198	93.0

TRISOMICS

Three trisomic plants were found in the same stock that had the reciprocal translocation and the mutant "Contabescent Anther." Two occurred among 182 offspring of 1 plant. The third was found among about 20 offspring from another plant. The populations were so uniform that the 3 trisomic plants, which were smaller than the others, were suspected of being haploid or otherwise abnormal. It is possible that other trisomics were present but did not affect the appearance of the plants sufficiently to attract attention. The 3 plants were about two-thirds the height of normal plants, were vigorous, and produced several tillers. There was no obvious morphological characteristic to distinguish them from one another or from normal plants. Careful observations on more individuals may reveal identifiable peculiarities.

There is probably no causal relation between the translocation previously described and the trisomes. If there had been a 3 : 1 distribution of the chromosomes at meiosis in a ring-forming plant, the offspring receiving the extra chromosome would have a chain of five chromosomes in some pollen mother cells. No more complex association than a trivalent was observed in the trisomic plants, but since the translocation apparently involves quite small segments it is difficult to exclude the possibility that chains of five do occur.

Cytologically there was no clue as to whether the same chromosome was involved in the three trisomic plants. Cytological observations were limited, however, to meiotic stages, where chromosome morphology is not very well expressed. Further observations on root tips or microspores should make it possible to determine whether either of the satellited chromosomes as shown in plate 1, *E*, and by Lewitsky (*10*) is present in triplicate.

In some pollen mother cells the chromatids of the extra chromosome separated in the first division. These presumably accounted for the lagging chromosomes at the second division and for most of the micronuclei in the quartets.

Photomicrographs (pl. 1, *F* and *G*) show typical configurations of meiosis in the trisomic plants. Table 4 summarizes the observations on the extra chromosome in two of the plants. A considerable proportion of the quartets had no micronucleus and approximately one-third of the spores probably had eight chromosomes (calculated from table 4). There was no observed cause for the considerable number of quartets in plant 202 1 with more than two micronuclei. Four quartets in this plant had five micronuclei each; others had three or four.

The chromosomes of a trisome in trisomic plants are known to tend to "misdivide"; i. e., to divide transversely instead of longitudinally across the centromere. As a result of this "misdivision" half chromosomes are formed with terminal centromeres.[7] This sort of behavior was observed in several pollen mother cells in the trisomic barley plant 202-2. Plate 1, *H*, shows a chromatid after a "misdivision." The irregularity in this case probably occurred at the second anaphase, though in another cell at second metaphase two fragments were already present so the "misdivision" in this case presumably had occurred in the first division. From results reported in other organisms, half chromosomes with terminal centromeres and secondary trisomes might be expected if sufficiently large progenies from the trisomic plants were examined.

Trisomic offspring have been obtained from two of the three original plants (202-2 and 207-3).

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[7] See Rhoades (*14*) for a review of the literature relating to this phenomenon

TABLE 4.—*Observations on meiosis in trisomic plants*

Plant	First metaphase		First anaphase		First telophase			Second anaphase			Second telophase			
	Cells with trivalents	Cells with univalents	Cells with chromatids separating	Cells with chromatids not separating	Cells with indicated number of micronuclei			Cells with indicated number of lagging chromosomes			Quartets with indicated number of micronuclei			
					0	1	2	0	1	2	0	1	2	3 or more
	No	*No*	*No*	*No*	*No*	*No*	*No*	*No*	*No*	*No*	*No*	*No*	*No*	*No*
202-1	4	43			41	4	0				95	40	62	20
202-2	12	25	14	14	100	12	1	5	2	2	33	13	10	0

TETRAPLOIDS

Three tetraploid stocks were available for study, but because of the lack of time and the abundance of other material only limited observations were made on them. One stock, "Big Boy," was found in 1936 by H. V. Harlan [8] in a selection from the cross Trebi × Club Mariout; another was a tetraploid strain of Opal B [9]; the third stock was a tetraploid line of the variety Everest, obtained by Harland Stevens [10] from a treatment of germinating seeds with colchicine

There was no conspicuous difference in the meiotic behavior of 4*n* plants from the three sources. Quadrivalents were the rule (pl. 1, *I*) Univalents and trivalents were fairly common, but micronuclei in the quartets were not numerous.

Each of the three lines had occasional small plants; some were extreme dwarfs, forming rosettelike clumps. Some of the less abnormal plants were found to have 26 or 27 chromosomes, but no count was obtained on the more extreme dwarfs.

Stevens had planted seeds from a few small plants of the 4*n* Everest stock, and in turn the small plants in these progenies were examined. One of the smaller plants had 29 chromosomes, another 29 or 30, and a third had 28. This last plant possibly had an unbalanced constitution, with more than 4 homologous chromosomes in some cases and fewer than 4 in others.

Six plants that grew from attempted crosses of diploid pollinated by tetraploid had 14 chromosomes at meiosis. One plant from the attempted cross of tetraploid by diploid had 28 chromosomes in a root tip.

Table 5 presents data on the fertility of the three tetraploid strains. Although the data on corresponding diploid lines are not available, it

TABLE 5.—*Fertility of tetraploid barleys*

Tetraploid strain	Location where grown	Year	Florets	Seed	Fertility
			Number	*Number*	*Percent*
Big Boy	Aberdeen, Idaho	1936	193	123	63 7
Do .	Madison, Wis	1940	153	70	45 8
Opal B	.do .	1940	64	26	40 6
Do	Sacaton, Ariz	1940	120	42	35 0
Everest .	Aberdeen, Idaho	1940	357	160	44 8

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[8] Principal agronomist, Division of Cereal Crops and Diseases

[9] Obtained from A. Müntzing, of Svalof, Sweden

[10] Assistant agronomist, Division of Cereal Crops and Diseases.

can be safely assumed from this table that fertility in tetraploids is considerably lower than in the diploids.

OBSERVATIONS ON MISCELLANEOUS MUTANTS AND HYBRIDS IN BARLEY

Seven mutants (in addition to "Contabescent Anther") were examined for meiotic irregularities. No abnormality was observed at the stages examined, which in almost every case included first metaphase and anaphase, but the observations were of necessity limited. The mutants included: "Curly Awns," one plant; "Sterile Flowers," seven plants; "Sterile Flowers, Smooth Stigmas," one plant; "All Wrong" (a dwarf), three plants; "Mary's Dwarf," one plant; "Multan" (a variety), two plants; "Uniculm," two plants; Suneson's "Male Sterile" (*20*), three plants.

In a search for trisomics, triploids, and haploids, offtype plants were sampled among the large number of varietal hybrids in the breeding project at Aberdeen. No chromosomal aberrations were found though 29 peculiar plants were examined.

SUMMARY

About 20 percent of the pollen mother cells in two plants of one progeny of barley had a bridge or fragment or both at the first division. These configurations are interpreted as due to crossing over in a heterozygous inversion.

A reciprocal translocation is described. Limited observations indicate that in about 60 percent of the pollen mother cells, segregations of the chromosomes were disjunctional. Fertility was about 65-percent perfect in three ring-forming plants.

Three trisomic plants were found. Observations on meiosis in two of the plants are recorded.

Observations are reported on meiosis, fertility, and chromosome number in three strains of tetraploid barley.

Examination of a number of mutants and varietal hybrids revealed a meiotic disturbance as characteristic of only one mutant.

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